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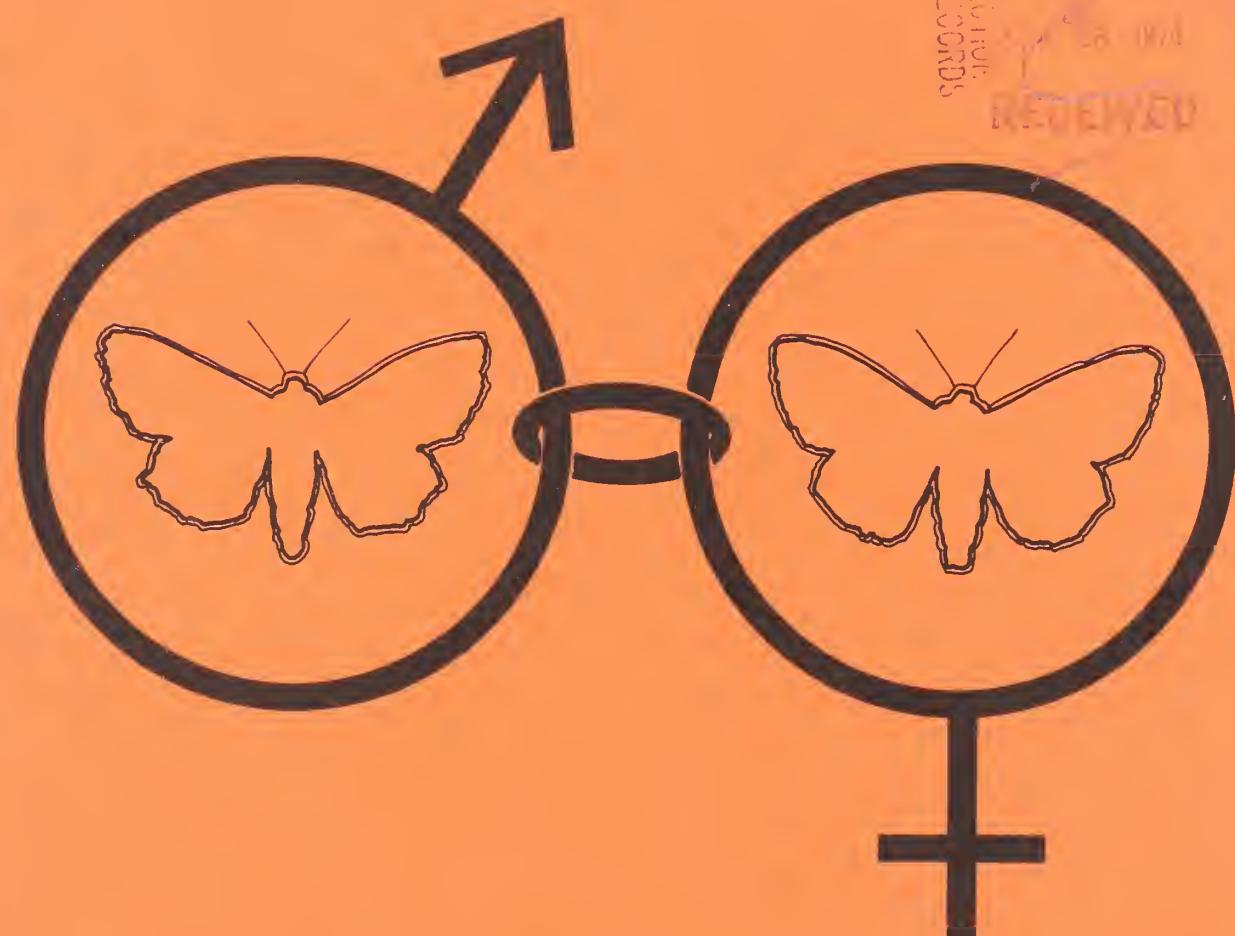
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INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY  
RESEARCH LABORATORY

Gainesville, Florida

FIRST SEMI-ANNUAL REPORT - 1974



AGRICULTURAL RESEARCH SERVICE  
U. S. Department of Agriculture

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FIRST SEMI-ANNUAL REPORT

1974

*Insect Attractants, Behavior, and Basic Biology*

*Research Laboratory*

*USDA-ARS, Southern Region, Florida-Antilles Area*

*P. O. Box 14565*

*Gainesville, Florida 32604*

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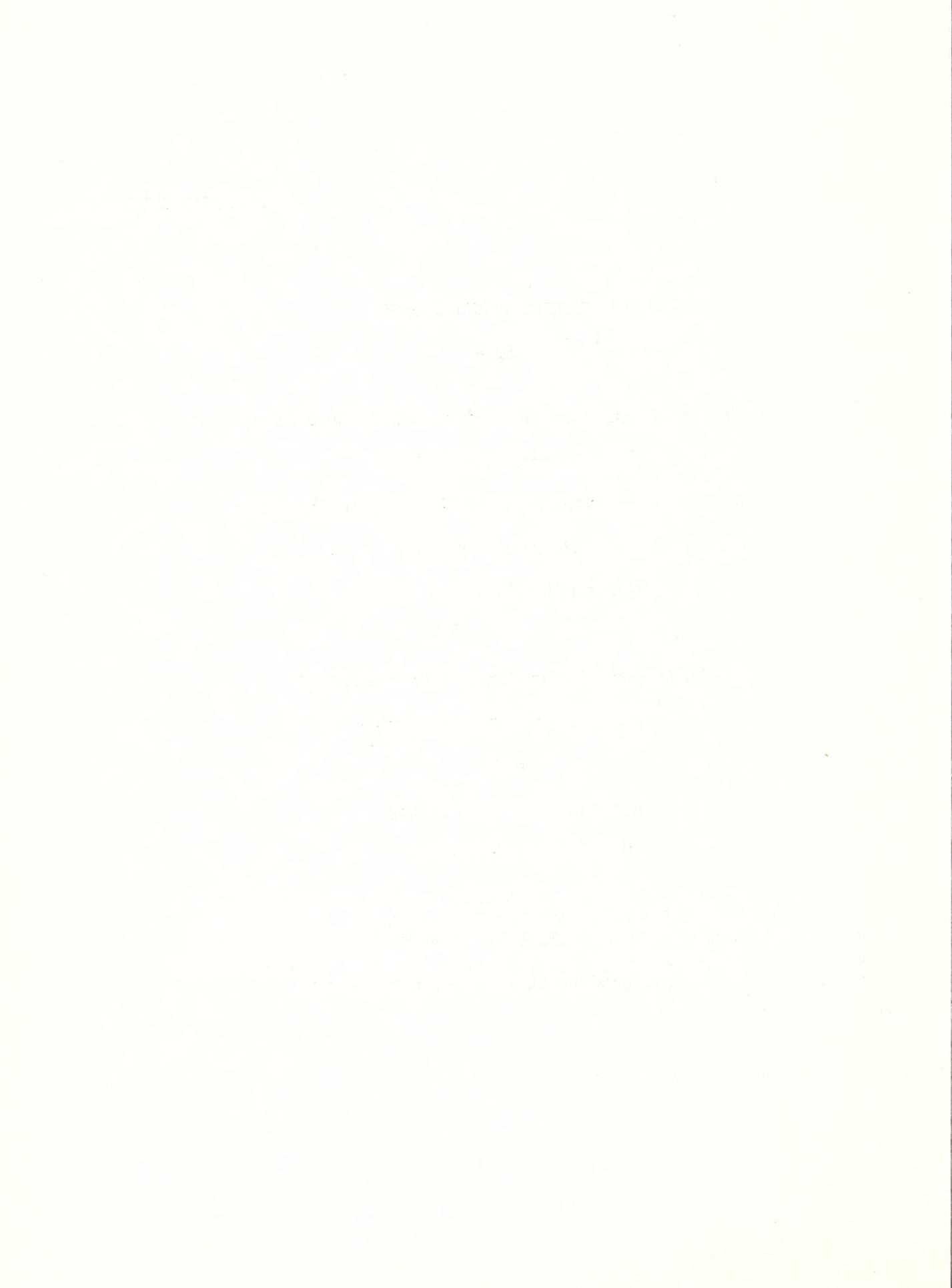
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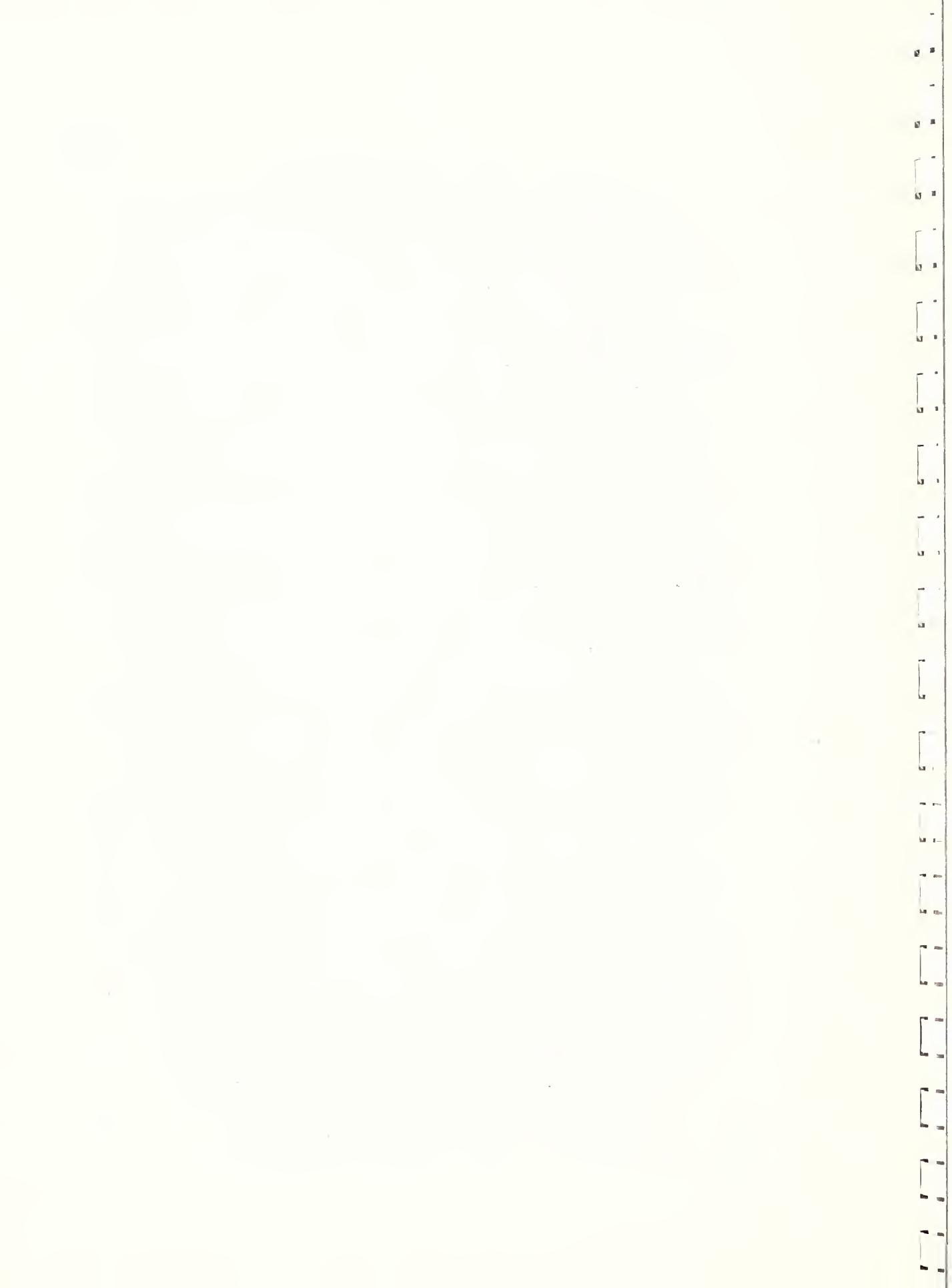
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Growth and Development

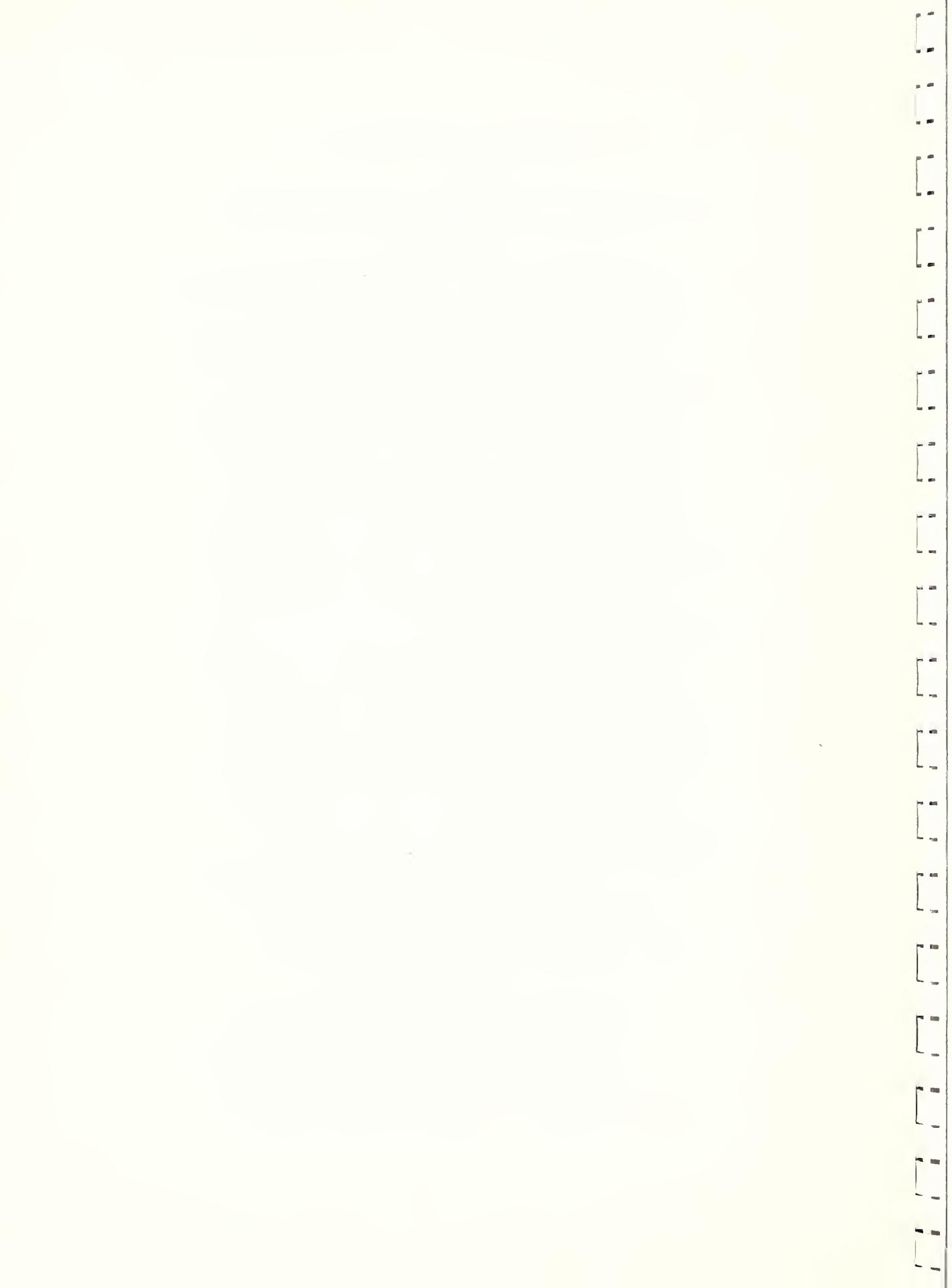
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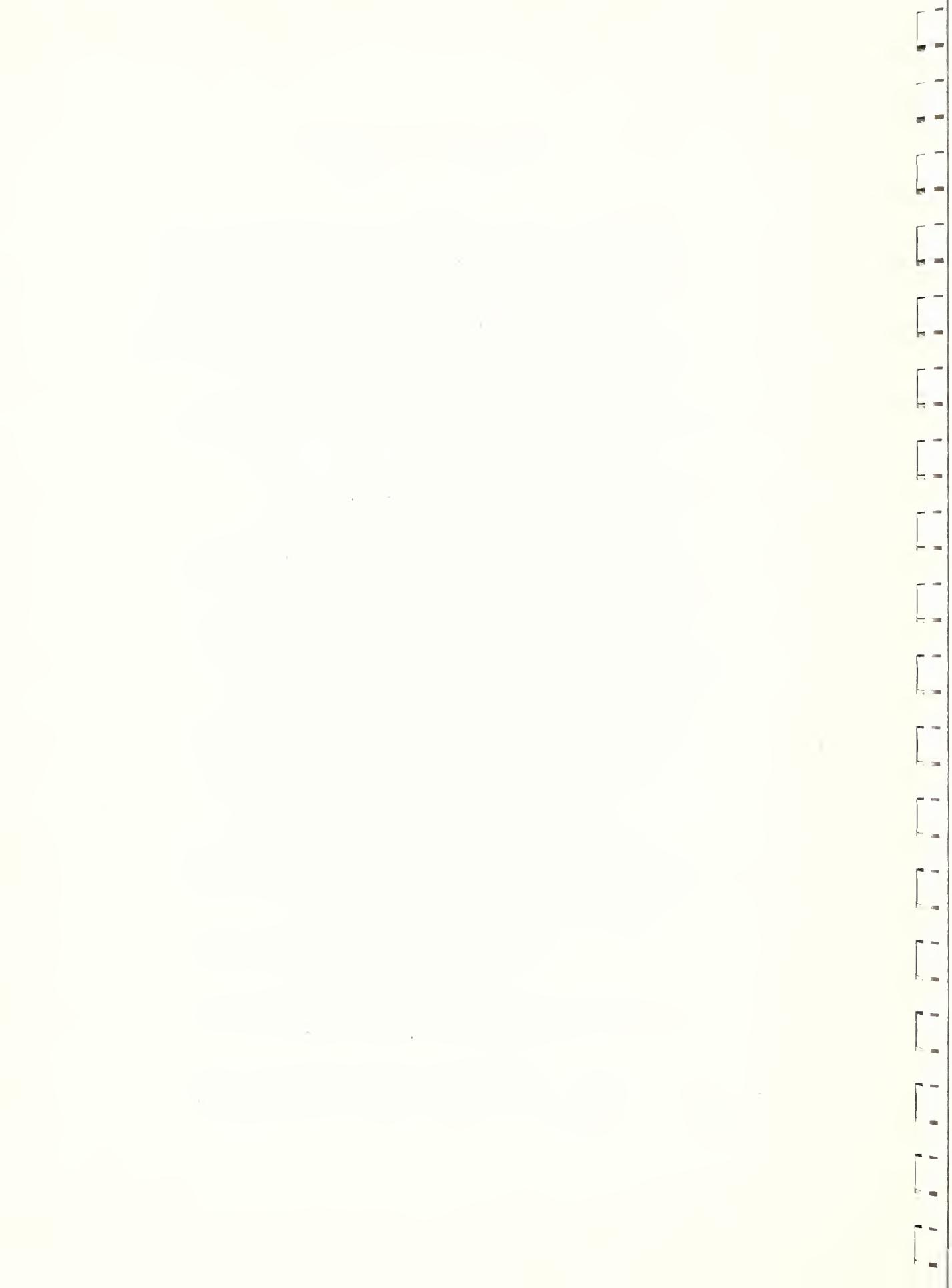
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Effect of Juvenile Hormone and Its Mimics on Michondrial  
Metabolism in the Indian Meal Moth,

Plodia interpunctella

Part I: Mechanism of Action

D. L. Silhacek and K. Kohl

(Continuation of report 02 73(7-12))

Objective: In studies on the effects of hormones on intermediary metabolism of Indian meal moths, we found that, in vitro, juvenile hormone (JH) inhibits some mitochondrial oxidations while stimulating others. These results suggested that the JH effects on mitochondrial metabolism might provide valid criteria for evaluating potentially active JH-mimics. The purpose of the present experiments is to determine the biochemical mechanisms of the JH actions.

Methods: Mitochondria were isolated from Indian meal moth larvae by differential centrifugation. Mitochondrial oxidative activities with succinate and pyruvate-malate as substrates were determined with a vibrating platinum electrode. Initial experiments were conducted with mitochondria isolated from larvae of different known ages. Subsequent experiments were conducted with mitochondria isolated from newly molted last-instar larvae.

Results: Studies on the JH stimulation of succinate oxidation in isolated mitochondria were continued. Two naturally-occurring hormones, JH-I and JH-II, were extremely effective in stimulating succinate oxidation. The other naturally-occurring hormone, JH-III was not effective. The methyl ester of linolenic acid, but not the esters of oleic or linoleic acids, was effective in stimulating succinate oxidation. JH-I was the only one of these compounds that was effective in inhibiting NADH-linked oxidations in mitochondria. These data strongly support the contention that the observed effects of JH on mitochondria are highly specific effects required for normal insect development and are not non-specific artifacts of an in vitro system.

Plans: Studies will continue on determining what molecular structural characteristics are needed for affecting mitochondrial metabolism and elucidating the mechanism of JH-stimulated succinate metabolism.



Effect of Juvenile Hormone and Its Mimics on Mitochondrial  
Metabolism in the Indian Meal Moth, Plodia interpunctella  
Part II: Metabolic Effects

D. L. Silhacek and D. Firstenberg

(Continuation of report 02 73(7-12))

Objective: In studies on the effects of hormones on intermediary metabolism of Indian meal moths, we found that, in vitro, juvenile hormone (JH) inhibits some mitochondrial oxidations while stimulating others. These results suggested that the JH effects on mitochondrial metabolism could have profound effects on the overall metabolism of the insect. The purpose of the present experiments is to investigate possible effects of JH on the metabolism of the insect.

Methods: Mitochondria were isolated from Indian meal moth larvae by differential centrifugation. Mitochondrial oxidative activities with succinate and pyruvate-malate as substrates were determined with a vibrating platinum electrode. Cytochrome content was measured spectrophotometrically. Experiments were conducted with mitochondria isolated from larvae of different known ages.

Results: The previously observed effects of JH on mitochondrial oxidations suggested that one metabolic effect could be to alter the Kreb cycle steady state so that porphyrin synthesis was increased. This hypothesis was tested by measuring mitochondrial cytochrome (formed from porphyrins) content in mitochondria isolated from JH-treated and untreated larvae. The results of these experiments definitely demonstrate that JH stimulated cytochrome synthesis. Experiments still in progress suggest that other hormonal factors may participate in this regulation of porphyrin metabolism.

Plans: Studies will continue on determining the role of JH-induced mitochondrial metabolic changes in influencing the metabolism of the insect.



In vitro Bioassay of Insect Hormones

H. Oberlander and C. E. Leach

(Continuation of report 07 73(7-12))

Objective: To develop sensitive and fast bioassay procedures for structure-activity studies of insect hormones and hormone mimics. Compounds which may have ecdysone, anti-ecdysone or juvenile hormone activity will be evaluated by an in vitro bioassay system.

Methods: Wing discs of the Indian meal moth, Plodia interpunctella (Hübner), are incubated in vitro in modified Grace's tissue culture medium with the candidate hormonal agent. The ability of the compound to stimulate or inhibit cuticle deposition is noted.

Results: The effects of 4 synthetic juvenile hormone analogues on cuticle deposition were evaluated. ZR 515 was the most active, followed by ZR 219 and ENT 20119a. ZR 512 did not inhibit cuticle deposition in vitro even at 1000 ppm. Another compound that was lethal to larvae (presumably by interfering with cuticle formation) was PH 60-40. However, this agent did not prevent cuticle deposition in vitro.

Plans: New compounds that are thought to act by interfering with cuticle formation or hormone action will be tested as they became available.



Exposure of Stored-Product Coleoptera to  
Hormone-Treated Culture Medium

F. O. Marzke and W. G. Sercey

(Continuation of report 04 73(7-12))

Objective: To determine the effect on development of exposure of the cigarette beetle, (Lasioderma serricorne) or red flour beetle, (Tribolium castaneum) to culture medium treated with juvenile hormone-mimicking compounds.

Methods: Cigarette beetle and red flour beetle culture medium (flour, corn meal, and brewers yeast) was treated with juvenile hormone-mimicking compounds at 0.5-250 ppm or higher. Eggs, last instar larvae or adults were placed in the treated medium and the subsequent development of the insects observed.

Results: Media aged 2 years after treatment with Zoecon 515 at 10 ppm or higher were still effective in preventing adult emergence when last-instar larvae of the red flour beetle were exposed to the treated diet.

Zoecon 619 at 10 ppm or higher prevented adult emergence of the red flour beetle and at 2.5 ppm or higher adult emergence of the cigarette beetle.

Plans: Effectiveness of additional juvenile hormone-mimicking compounds in preventing development of stored-product Coleoptera will be determined. The effects of these compounds on insect behavior and fertility will be investigated.



## Exposure of Stored-Product Coleoptera to Hormone-Treated Papers

F. O. Marzke and W. G. Sercey

(Continuation of report 05 73(7-12))

Objective: To determine whether confinement to surfaces treated with juvenile hormone-mimicking compounds would prevent development of the cigarette beetle, (Lasioderma serricorne), and what are the factors contributing to this effect.

Methods: Whatman No. 1 filter papers were treated with an acetone solution of juvenile hormone-mimicking compound Zoecon 515 at 10, 30, 100, or 300  $\mu\text{g}/\text{cm}^2$ . Five- to 7-day-old mated or unmated cigarette beetle adults were confined to the surfaces for 2 $\frac{1}{2}$  hrs or less and then transferred to vials containing untreated culture medium. The numbers of eggs, larvae, or emerged adults were then determined.

Results: Exposing cigarette beetle males to pheromone-treated paper before mating had little effect on the  $F_1$  generation.

Exposing mated cigarette beetle females to culture medium for a day before exposure to hormone-treated paper did not negate the effect of the treatment; i.e., most eggs laid after exposure to the treatment did not hatch.

In the attempt to determine at what stage in the reproductive cycle of the female cigarette beetle the effect of exposure to hormone occurred several tests were initiated: (1) Females were mated 0-4 days after exposure, and (2) Females were remated 3 days after exposure to the treated paper. Remating 3 days after exposure resulted in the oviposition of eggs that hatched. Results from other tests are incomplete.

Some  $F_1$  adults emerged when female cigarette beetles were exposed to papers treated at 100  $\mu\text{g}/\text{cm}^2$  or less for 16 hours or less; i.e., a  $F_1$  adult reduction of 75% or more resulted from an 8-hr exposure to 30  $\mu\text{g}/\text{cm}^2$ .

Tests in which mated cigarette beetle females were confined for various periods of time in empty jars before being placed in culture medium are still in progress.

Plans: Tests will continue to determine at what stage in the reproductive cycle the effect of juvenile hormone-mimicking compounds occurs.



Effect of Altosid 5E and ZR-619 5E on the Development of the  
White Peach Scale, Pseudaulacaspis pentagona (Targ.)

T. R. Ashley and T. C. Carlyle

Objective: To determine the effect of these 2 growth regulators on the development of the white peach scale.

Method: Concentrations of 0.01, 0.12, and 0.24% of each compound were sprayed to the point of run off on the various stages in the scale's life cycle. Potato tubers served as host for the scale. The amount and distribution of mortality are recorded and any abnormal growth patterns are photographed through a scanning electron microscope.

Results: Only settled crawlers have been sprayed with these compounds. Altosid 5E is more active than ZR-619. The 0.12 and 0.24% solutions of Altosid and the 0.24% solution of ZR-619 killed the highest percentage of crawlers during the 1st 7 days after application while the 0.01% Altosid and 0.01 and 0.12% ZR-619 solutions caused the highest mortality 2 to 3 weeks after application. With the exception of the 0.12% ZR-619, all of the solutions tested killed 100% of the crawlers. The 0.12% ZR-619 has destroyed 96.5% during a 35-day period.

Plans: These solutions will be tested against the remaining stages of the scale's life cycle. Lower concentrations of both compounds will be tested on the crawler stage.



Metabolism of Alpha- and Beta-ecdysone in Plodia interpunctella

D. L. Silhacek, H. Oberlander, and S. Carlyle

(Continuation of report 08 73(7-12))

Objectives: Previous studies have provided considerable insight into the physiological mechanism of ecdysone action on cultured wing disks. Meaningful interpretation of these and other findings requires an understanding of the metabolic fate of ecdysone in the insect tissues. The purpose of this study was to determine whether ecdysone is metabolized during incubations with wing disc tissue and with subcellular fractions isolated from larvae.

Methods: Wing discs were dissected from Indian meal moth larvae and cultured by established methods. Subcellular fractions of whole larvae were prepared by modifying methods. Procedures for the quantitative extraction, separation, and measurement of ecdysone and its metabolites were developed using isotopically labeled hormone.

Results: Procedures for the isolation and purification of enzymatically active microsomal fractions from Plodia larvae and systems for measuring NADH and NADPH oxidations in microsomes have been developed. However, the enzymatic mechanisms of the microsomal oxidations are not yet understood. Juvenile hormone and its mimics inhibit these microsomal oxidations. Several insecticides had a similar effect.

In a related study, the metabolism of the ecdysones by Plodia wing discs and fat body is being studied. Cultured wing disks and fat bodies metabolized alpha-ecdysone but do not appear to convert alpha-ecdysone to beta-ecdysone.

Plans: Studies will continue on elucidating the enzymatic pathways of microsomal oxidations and on the metabolism of the ecdysones by cultured insect tissues.



Hormonal Control of Chitin Synthesis In vitro

H. Oberlander and C. E. Leach

(Continuation of report 09 73(7-12))

Objective: We have developed a model system to investigate the mode of action of ecdysone and juvenile hormone on the initiation and inhibition of metamorphosis. Ecdysone stimulates and juvenile hormone inhibits cuticle deposition in imaginal discs in vitro. We are focusing on the action of these hormones on the biosynthesis of chitin.

Methods: Wing discs of the Indian meal moth, Plodia interpunctella (Hübner), are cultured in vitro in a modified Grace's medium. The incorporation of D-glucosamine-6-H<sub>3</sub> and D-glucose-6-H<sub>3</sub> into the discs is evaluated. The effects of inhibitors of chitin synthesis are evaluated.

Results: The inhibitory action of juvenile hormone is being compared to other possible inhibitors of cuticle deposition of known mode of action. Cytochalasin B inhibits glucose transport in many animal systems. If it also inhibits glucose uptake by disc cells, then chitin will not be made unless there is already a sufficient precursor pool within the cells. Beta-ecdysone caused a 300% increase in glucose uptake by cultured imaginal disc cells. This increase in uptake was totally inhibited by a concentration of cytochalasin B which prevented cuticle formation. Thus, the disc cells do require an extrinsic supply of chitin precursor at the developmental stage taken for tissue culture.

Plans: This in vitro system will continue to be used to investigate the mode of action of juvenile hormone. We will determine at what level (precursor uptake, synthesis, deposition) juvenile hormone acts to prevent ecdysone-induced cuticle formation in vitro.



Control of Egg Maturation in Dermestid Beetles

K. Vick and J. Coffelt

(Continuation of report 10 73(7-12))

Objectives: To elucidate the hormonal control of egg maturation in dermestid beetles, including the role of the male in stimulating egg production.

Methods: A combination of electron microscopy and micro-chemical analysis of the female hemolymph and developing oocytes is being used to study the control of vitellogenesis in the black carpet beetle.

Results: Studies are still in progress and no firm conclusions have been reached.

Plans: Studies are being conducted to elucidate the biochemical steps involved in pre-mating and post-mating vitellogenesis.



Dissociation and Reaggregation of  
Fat Body Cells During Metamorphosis

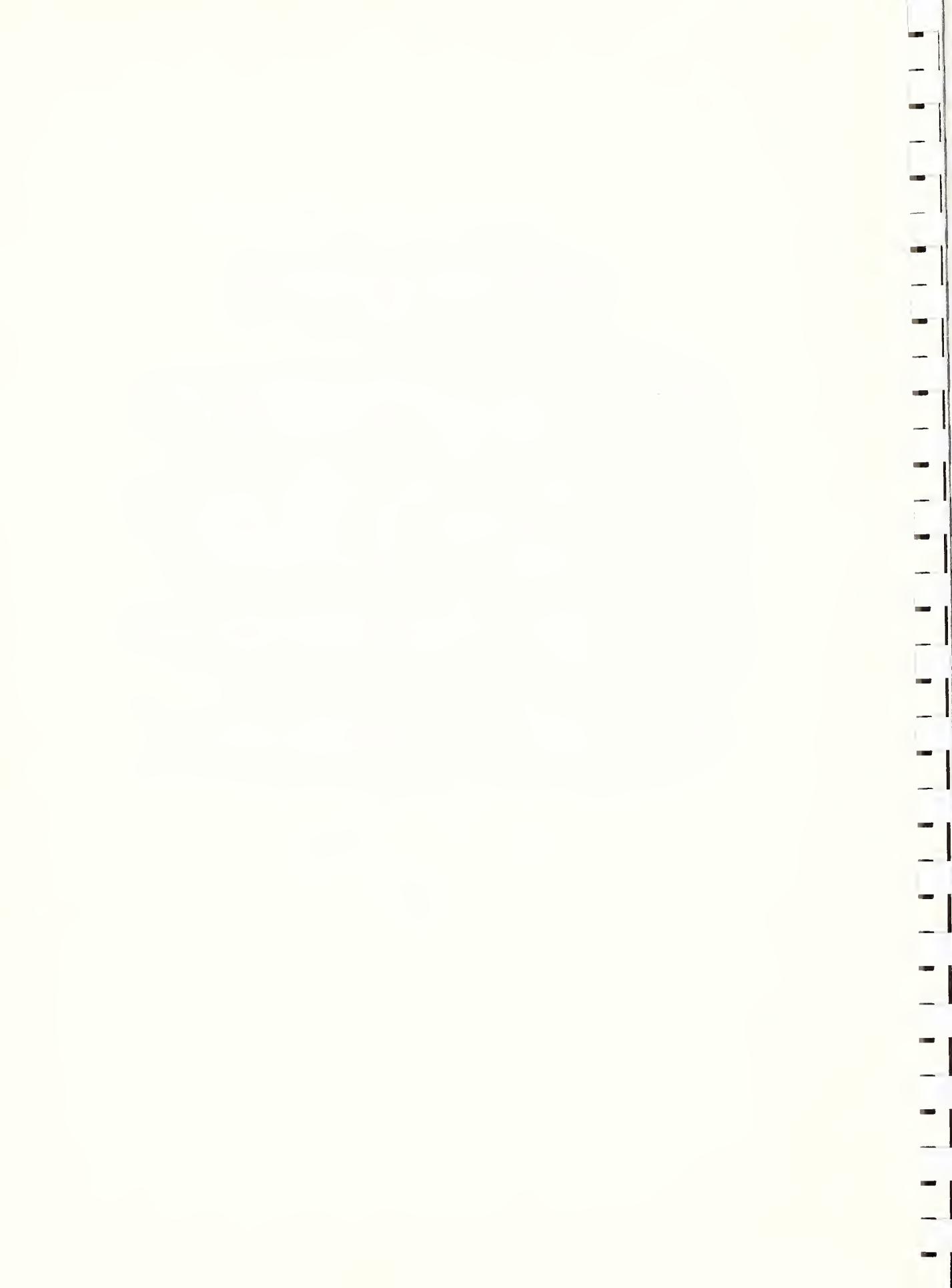
H. Oberlander

Objective: To determine the mechanisms which control dissociation and reaggregation of fat body cells during metamorphosis. To evaluate this system as a new model for investigating hormone action.

Methods: Fat body cells of larvae of the Indian meal moth, Plodia interpunctella (Hübner), are cultured in vitro in a modified Grace's medium. The degree of dissociation or reaggregation is monitored by microscopic examination.

Results: Beta-ecdysone induces complete dissociation of fat body cells in vitro. The hormone is equally effective with fat body taken from the early, middle or late part of the last larval stadium.

Plans: The requirements for reaggregation of fat body cells in vitro will be investigated. The effects of juvenile hormone on dissociation and reaggregation will be examined.



Reproductive Biology of the Cigarette Beetle,  
Lasioderma serricorne (Fabr.)

J. A. Coffelt

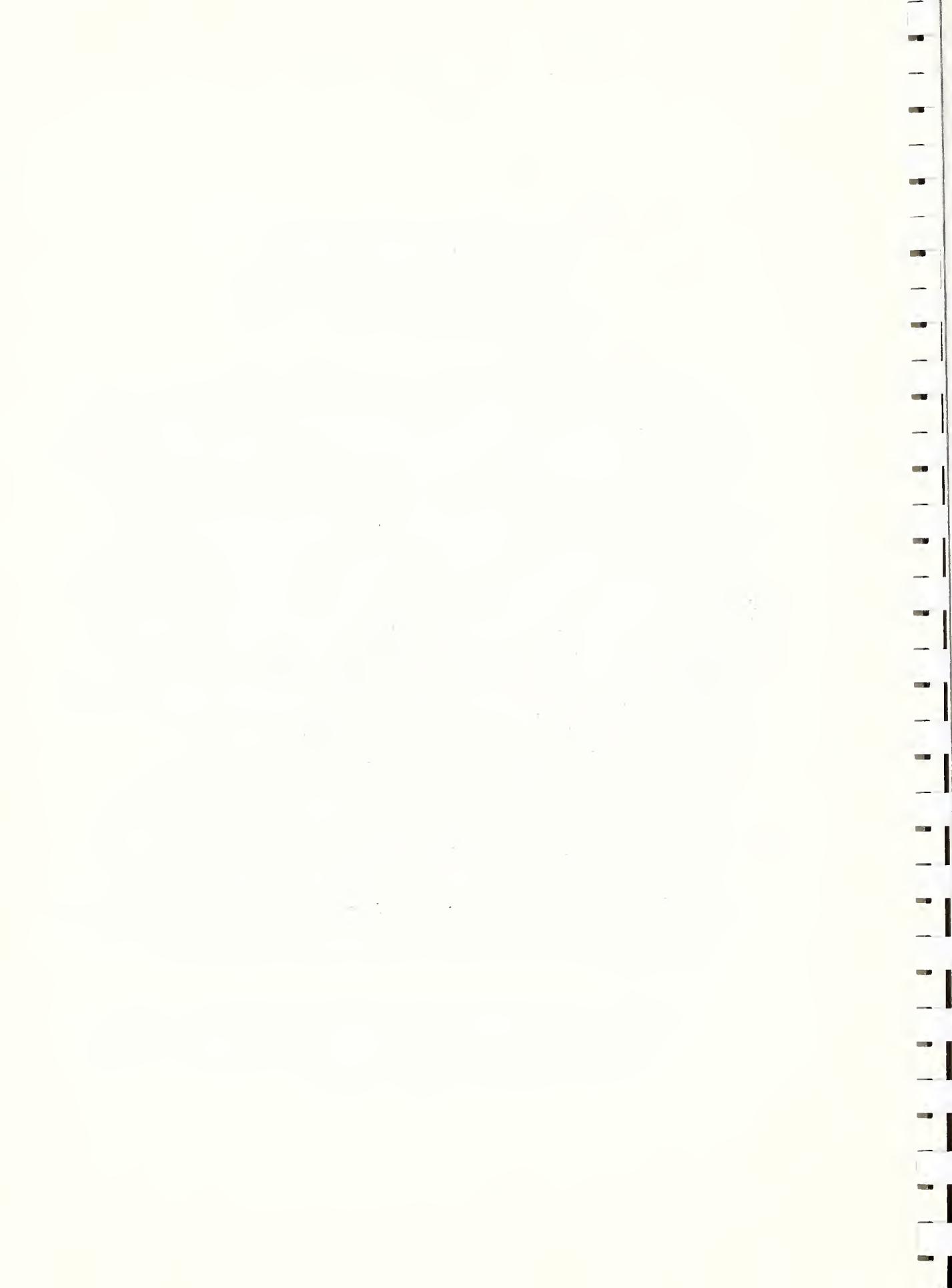
(Continuation of report 12 73(1-6))

Objectives: These studies are part of a continuing series of investigations of the reproductive biology of the cigarette beetle and have as their specific objectives: 1) The determination of the pattern of sperm utilization among multiple-mated females and 2) the determination of the relationship between multiple mating and female reproductive potential.

Methods: Patterns of sperm utilization were determined by the use of the wild-type(+) and black(b) strains. Single-pair matings, + x + and b x b, yielded 2 groups of females for test purposes. Each female was allowed 3 days for initial oviposition. Subsequently, wild-type females were remated to black males and black females were remated to wild-type males. The occurrence of brown (heterozygous) individuals among the progeny produced following the second mating was taken as evidence of the utilization of sperm from that mating. Remated females were returned to the incubator (fresh oviposition medium) and not further disturbed. Progeny counts were made ca. 6 weeks later. In addition, phenotypes of all offspring were recorded.

Results: There was no significant difference between the numbers of offspring produced by once- or twice-mated females. Among wild-type females, about 80% of the progeny produced subsequent to the second mating were heterozygous. In the reciprocal series of crosses approximately 90% of the offspring produced following the second mating were derived from sperm received at the second mating. Analyses of the data are incomplete as of this writing, but it is evident (4 reps) that multiple mating has little or no effect upon overall female fecundity and that multiple mating may function largely or wholly as a mechanism to increase gamete recombination.

Plans: Studies will be undertaken to determine if there are detectable behavioral differences between wild-type and black strains. Attempts will be made to correlate willingness to remate with pheromone content among both parous and nulliparous mated females.



Reproductive Systems and the Mechanics  
of Copulation in Plecia nearctica

N. C. Leppla and T. C. Carlysle

(Continuation of report 58 73 (1-6))

Objectives: To describe the unique, evolutionarily intermediate reproductive systems of male and female "lovebugs"; and to study positioning of the internal morphological structures during copulation.

Methods: Males, females, and mating pairs of these bibionid flies were preserved and examined histologically or by dissection.

Results: Males produce a hyaline bilobed spermatophore that is contained within a sclerotized muscular "ejaculatory pump." During coition, the spermatophore is partially extruded and appressed into the "pelvis-like" genital atrium of the female. Females cooperate in clasping and direct the distal portion of the spermatophore. Ambiguities between observed behavior and existing anatomical relationships resulted in previously reported erroneous mating frequencies. Male genitalia are torsioned, but intromission, even though superficial, is mesal not lateral. Sperm is actually transferred by means of a gelatinous filament that extends from the spermatophore, branches into both spermathecal ducts, and terminates in the bursa copulatrix. Sperm is forced from the male by a sclerotized protuberance that projects into the spermatophore as the "pump" contracts. After mating, branched filaments remain in the female, while the spermatophore and a portion of the main filament are retained briefly by the male. Construction and extension of the filaments, apparently a time-consuming procedure, and problems associated with disengagement could account for prolonged copulation in this species.

Plans: A manuscript is being prepared currently and no further research is warranted.



Analysis of Methods Used in Laboratory Culture of Opius longicaudatus

P. D. Greany, T. R. Ashley, and D. L. Chambers

(Continuation of report 13 73(7-12))

Objectives: To optimize biotic and abiotic conditions affecting laboratory rearing of Opius longicaudatus, a parasite of tephritid fruit fly larvae.

Methods: The effect of the host's diet upon parasite developmental time, pupal survivability, adult survival, and fecundity was analyzed. Bagasse and corn cob diets were tested. Parameters measured for non-parasitized bagasse and corn cob hosts included larval developmental time, pupal weight, percent emergence, and sex ratio of flies.

A second experiment was conducted to evaluate the influence of alternate collection and transfer methods upon the survival and fecundity of Opius longicaudatus. Methods used included anaesthesia by  $\text{CO}_2$  and  $\text{N}_2$ , chilling, and aspiration.

Results: Although fecundity data has not yet been completely analyzed, there were few differences in results obtained with the two types of host diet. Larval mortality was greater for parasitized corn cob larvae, but pupal mortality was greater for bagasse larvae. Overall, there was no significant difference in the yield of parasites and flies from hosts fed either type of diet. Corn cob larvae required one day longer before they began to pupate. There was no significant difference in survival of adult parasites reared from either type of host. However, parasites not provided hosts lived significantly longer than those provided with hosts. These results indicate that either diet could be used satisfactorily, providing that fecundity values are also equivalent.

The study on alternate collection methods yielded no significant differences among methods tested, although fecundity analyses are yet to be completed. Brief anaesthesia with  $\text{CO}_2$  is now being used preferentially for routine culture work as it is the most convenient of the several methods tested.

Plans: Studies to establish the influence of crowding of caged parasites are planned. Varied host-parasite ratios will be evaluated with respect to actual and effective parasitization rates. Pupal holding conditions will be investigated.



Bacteremia as a Deterrent to Mass Rearing of Opius longicaudatus

P. D. Greany, G. E. Allen,<sup>1/</sup> B. Allen,<sup>1/</sup> and D. L. Chambers

(Continuation of report 14 73(7-12))

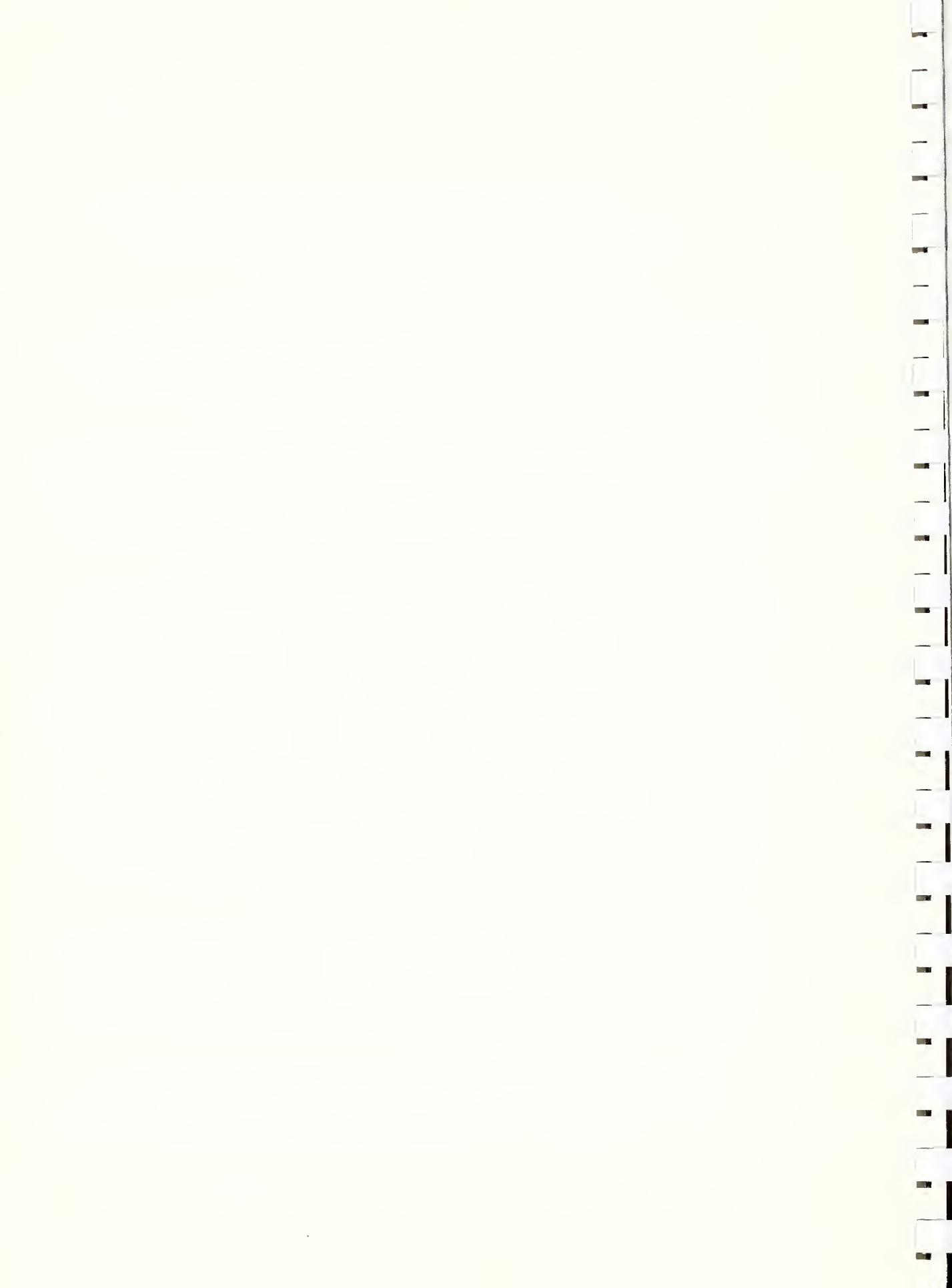
Objectives: To identify bacterial pathogens affecting developing and adult parasites, to describe the histopathology of affected stages, and to ameliorate the problem by use of selected antibiotics and/or optimized techniques.

Methods: Using methods described previously, further studies were performed to isolate potential bacterial pathogens from egg, larval, and pupal parasitized and non-parasitized hosts as well as adult parasites. Studies were performed to determine the effect of selected antibiotics in reducing mortality of parasitized hosts and adult parasites. Various modifications in culture methods were instituted so as to reduce the incidence of mortality.

Results: In addition to bacteria isolated previously, Proteus rettgeri was also found in association with dead parasitized pupae and adult parasites. Tests have been conducted on the use of Polymyxin B sulfate and Chloromycetin for control of bacterial pathogens. No significant reduction in mortality of parasitized hosts was observed as a result of treatment of host eggs or host larvae with these antibiotics. Pure cultures of Serratia marcescens, Proteus rettgeri, and P. morganii were found to be sensitive to methenamine mandelate, an inexpensive chemotherapeutic agent, and it is being added prophylactically @ 250 ppm to the larval rearing medium, and to the honey and drinking water provided adult parasites. High rates of mortality still occur, however. It is of interest to note that incorporation of antibiotics into the larval rearing media has not interfered with the growth of Caribfly larvae such as might be expected if obligatory symbiotes had been destroyed.

Indications exist that mortality may be due primarily to stress from adverse environmental conditions existing during particularly susceptible stages, allowing opportunistic microorganisms to invade host tissues. The bacteria isolated from parasitized hosts can also be found in association with apparently healthy, non-parasitized hosts, indicating that they may normally be innocuous.

Plans: Tests will be conducted to establish whether pathogens are vectored at the time of oviposition by parasite females. Histopathologic studies will be performed to identify specific ontogenetic stages at which bacteria invade host tissues. Correlated studies on environmental effects will be performed.



Introduction of Opium oophilus into Florida for Control  
of the Caribbean Fruit Fly

P. D. Greany, D. L. Chambers, and W. J. Schroeder<sup>1/</sup>

Objective: To establish Opium oophilus Full., a braconid parasite which effectively attacks the eggs of several important fruit fly species, in the laboratory and in the field as a parasite of Anastrepha suspensa (Loew), making it available as a control agent and as a target organism for research.

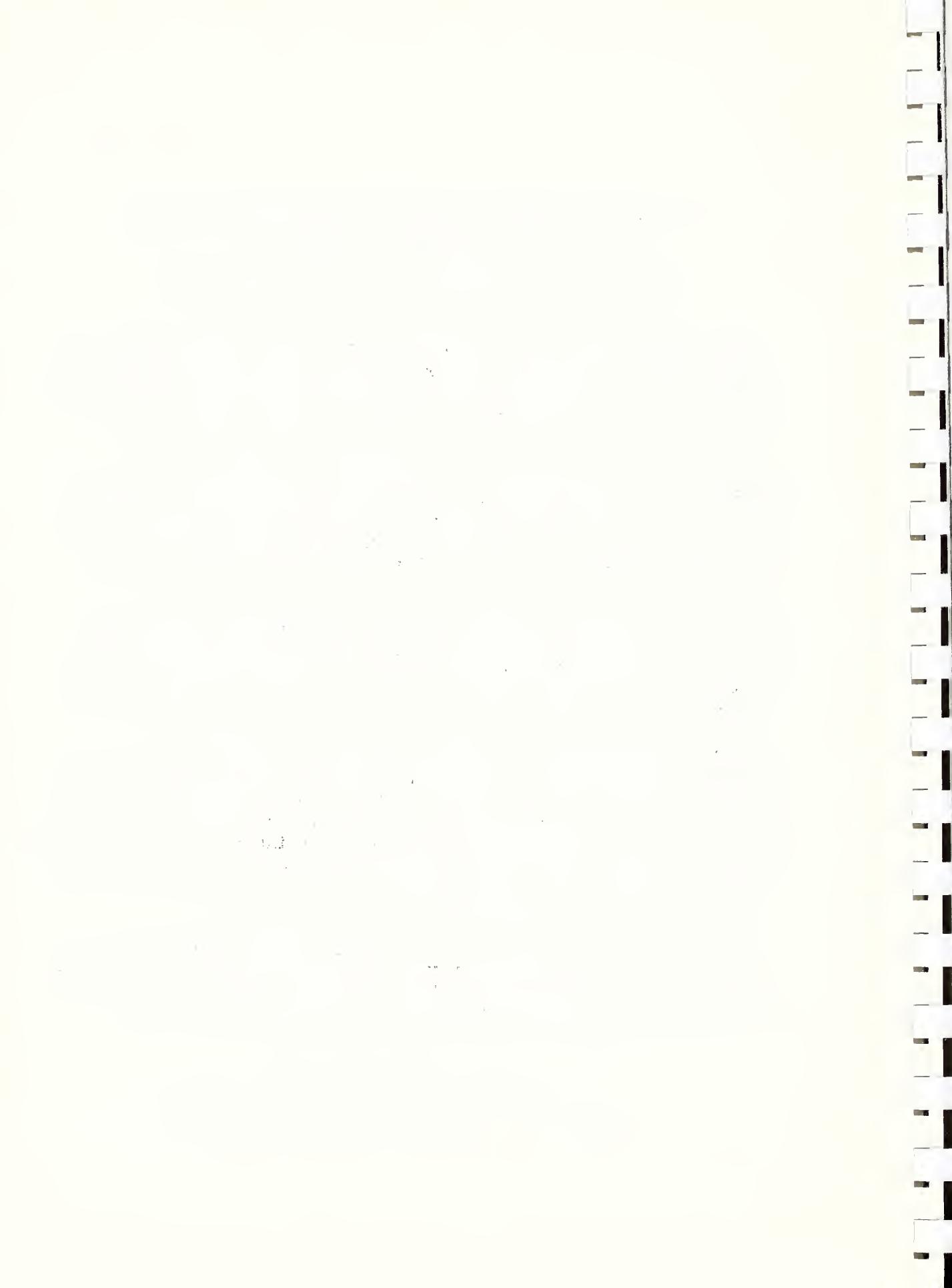
Methods: Adult O. oophilus males and females were field collected at the Waimanalo Experimental Farm on the Hawaiian Island of Oahu. They were shipped to Florida via expedited air handling and were processed in the quarantine facility of the Division of Plant Industry, Gainesville, Fla. Parasites were provided with fruits shortly after attack by Caribbean fruit flies.

Results: To date, 26 male and 47 female O. oophilus adults have been reared from the hosts exposed to parasitization, thus demonstrating the suitability of the Caribbean fruit fly as a host. Environmental conditions conducive to mating and permitting good survival have been satisfactorily attained.

Certain problems have been encountered in the effort to increase the size of the laboratory culture. It has been difficult to obtain fruit which is suitable for oviposition and development by the Caribfly, and in which host eggs are readily attacked by the parasites. These criteria have been met best by guavas, but guavas are available only intermittently in Florida. While other fruits such as peaches may serve as excellent hosts for the fly, O. oophilus females seldom attack eggs in such fruit, indicating a definite host plant involvement in host selection.

Plans: Attempts are being made to find either a suitable and easily obtained fruit as a substitute for guavas, or to create an artificial egging device suitable for oviposition by the fly and the parasite. Behavioral studies will be conducted toward this end in Florida and in Hawaii.

<sup>1/</sup> Research Entomologist, Hawaiian Fruit Flies Investigations Laboratory, Honolulu, Hi.



Calcium Alginate: A Gelling Agent in  
Diet for Lepidoptera

N. R. Spencer<sup>1/</sup> and N. C. Leppla

(Continuation of report 16 73(7-12))

Objective: To adapt calcium alginate as a substitute gelling agent in artificial diet for Lepidoptera.

Methods: A cooked diet containing carrageenan and an uncooked medium with calcium alginate substituted for this gelling agent were formulated, and tested on 4 generations of cabbage looper larvae.

Results: Insects reared on the 2 substrates exhibit statistically identical development (egg hatch, larval survival, pupal weight, adult yield, and fecundity). Diet is prepared by slowly adding calcium alginate to water and formalin in a high-speed blender capable of maintaining a full vortex. After gelling, the vortex dissipates and  $Na_4P_2O_7$  must be added within 10 seconds. As the vortex is reestablished, dry ingredients are added and mixed to the desired consistency. One gallon batch:

Water	3260 ml	Tetracycline	250 mg
Pinto beans	275 g	MPH	8 g
Yeast	125 g	Formalin	15 ml
Wheat germ	200 g	Casein	100 g
Ascorbic acid	6 g	$Na_4P_2O_7$	6 g
Vitamins	13.2 g	$CaSO_4$	40 g

Plans: A manuscript has been prepared and no further research is warranted.

<sup>1/</sup> Research Leader, Biological Control, Doyle Conner Bldg., Gainesville, Fla.



## Culturing the Lesser Peachtree Borer

A. Q. Antonio, J. R. McLaughlin, and N. C. Leppla

Objective: To alleviate storage and supply problems associated with using thinning apples for rearing the lesser peachtree borer by developing a larval diet composed entirely of commercially available ingredients.

Methods: Preliminary screening of diets presently in use at the facility resulted in the choice of the modified corn earworm diet for further evaluation. The diet is prepared in 3 gal. batches by adding the dry ingredients, except ascorbic acid, to warm demineralized water in a 5 gal. steam jacketed kettle:

Pinto beans (ground)	412 g	Vitamin mixture	30 ml
Wheat germ	200 g	(400 g Hoffmann- LaRoche/1000 ml H <sub>2</sub> O	
Brewer's yeast	125 g	Tetracycline	250 mg
Casein	100 g	Methyl P-	
Carrageenan	46 g	hydroxybenzoate	8 g
Water	2700 ml	Formalin	15 ml
Sorbic acid	4 g	(40% sol'n)	
Ascorbic acid	13 g		

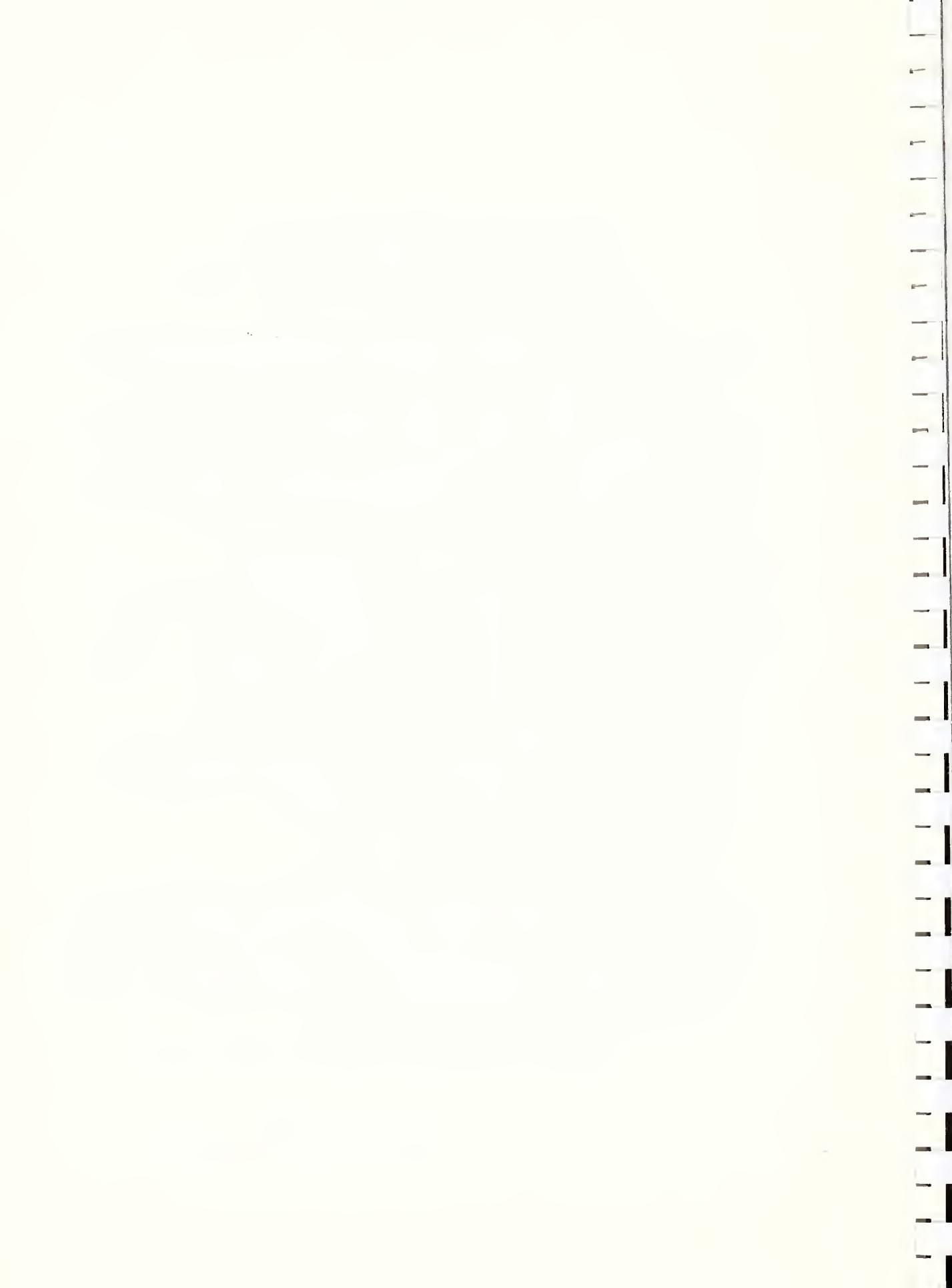
This mixture is heated at 78°C for 10 min; formalin is added after the first 5 min. Vitamins, tetracycline, and ascorbic acid are introduced with the hot medium to a high speed blender, mixed for 30 sec., and poured into 16 oz plastic cups.<sup>1/</sup> (10 oz diet/cup)

Several containers were also evaluated.

Results: The above diet produced insects in quantities and with development time and apparent size and vigor of adults in favorable comparison with the thinning apples. The suitability of a rearing container is always dependent upon local conditions and desired use of the insects. We found a 16 oz plastic container with cardboard lid most satisfactory. The diet was adopted as our rearing medium and we have 4th generation insects from the diet with no apparent decline in the colony.

Plans: No further research is anticipated beyond routine upgrading of procedure.

<sup>1/</sup> Plastic 16 oz cup (MC 160) with vented lid (LMC 61), Sweetheart Plastics, Inc.



Micro Techniques and Analytical Methods

R. R. Heath, J. H. Tumlinson, R. E. Doolittle, and A. Proveaux

(Continuation of report 18 73(7-12))

Objective: To develop analytical techniques for structural elucidation of microgram quantities of pheromones and other natural products and for analyzing and purifying naturally derived and synthetic compounds, particularly isomeric mixtures.

Methods: We are continuing to study  $^{13}\text{C}$  magnetic resonance spectroscopy (CMR) as a method for analysis and structure elucidation. High pressure liquid chromatography (HPLC) and GLC are also being used to analyze and purify pheromones. LC substrates obtained commercially and some prepared in our laboratory are being evaluated. New GLC liquid phases are being evaluated for isomer separation. Standards will be synthesized as necessary for this program.

Results: The CMR spectra of the 3,13-octadecadien-1-ol acetate isomers were used to determine that the Z,E-isomer contained about 5% of the E,Z-isomer. These two isomers have not yet been resolved by chromatography. Other Z,E-isomer pairs of acetates, aldehydes, and alcohols have been resolved using HPLC on silver nitrate coated silica gel. Silver nitrate substrates prepared in our laboratory are superior to those obtained commercially. A new GLC phase also shows promise in separating some isomeric pairs. A paper by Tumlinson and Heath was presented at the Third International Congress of Pesticide Chemistry (IUPAC), Helsinki, Finland, July 4, 1974.

Plans: Work is continuing to separate isomer pairs such as the (E,Z)- and (Z,E)-3,13-octadecadien-1-ol acetates by chromatography. Also CMR spectroscopy is continually being studied for its usefulness in solving our more complex problems.



Sex Pheromone Studies of the Navel Orangeworm

J. A. Coffelt, K. W. Vick, and L. L. Sower

(Continuation of report 22 73(7-12))

Objectives: To isolate and identify the female sex pheromone of the navel orangeworm and to develop an understanding of the physiological, behavioral, and environmental factors which may influence male response to, or female production of the pheromone.

Methods: The monitoring bioassays and general isolation procedures employed have been previously described. In addition, pheromone was also collected from females by 2 other methods. The first involved the brief rinsing of the female pheromone gland in ether. The second method consisted of obtaining ethereal rinses of the inner walls of jars in which females had been held for varying periods of time. Gland dips were made at the time of night corresponding to maximum female calling. The crude extracts (dip and jar wash) were assayed in serial dilution and analyzed by gas chromatography. The retention characteristics of the pheromone (dip only, 3 reps) were determined by means of preparative gas chromatography. Initial purification of the extracts (dip and jar wash) was obtained by means of liquid chromatography. Active fractions from the latter were analyzed by gas chromatography and bioassayed in serial dilution.

Results: Male response, in terms of female equivalents, to the gland dip preparations was quite similar to that previously reported from extracts of female abdominal tips, or of whole females. Detectable quantities of at least 2 compounds were consistently associated with biological activity in crude extracts, active liquid chromatography fractions, and the active fraction from preparative gas chromatography. Subsequent fractionation and bioassay suggested that one of the compounds is inactive.

Plans: We will continue to collect and process female pheromone by the above mentioned methods for the purpose of further analysis and isolation. Additional behavioral studies will be conducted, as needed, to improve the efficiency of pheromone collection techniques.



A Potent Sex Attractant for the Carpenterworm Moth,  
Prionoxystus robiniae

R. E. Doolittle, J. D. Solomon<sup>1/</sup>, W. L. Roelofs<sup>2/</sup>, M. Beroza<sup>3/</sup>,  
M. McKnight<sup>4/</sup>, and A. Tagestad<sup>5/</sup>

(Expansion and continuation of report 23 73(1-6))

Objectives: Isolate, identify and synthesize the female produced sex pheromone of the carpenterworm moth and evaluate its usefulness as a survey and/or control tool for the insect.

Methods: For isolation, identification, and bioassay methods, see (23 73(1-6)). Separation (purification) of the (Z,E) and (E,E) isomers to greater than 98% purity of 3,5-tetradecadien-1-ol acetate was accomplished by a combination of spinning band distillation and high pressure liquid chromatography.

Experiments designed to (1) extend the "field life" of the attractant; (2) determine the criteria of purity to be applied to the attractant; (3) determine optimum trap design and color, were conducted. A sufficient quantity of synthetic attractant (approx. 1 g.) was purified by the methods described above to conduct the tests in Stoneville and in North Dakota.

Results: The field control experiment has resulted in the trapping of thousands of male insects but no data on population reduction will be available until 1975. The field life of the synthetic attractant has been extended to three weeks. It has been determined that significant quantities of the (E,E)-isomer of 3,5-tetradecadien-1-ol acetate do not significantly alter the attractiveness of the (Z,E) isomer.

The aspen carpenterworm was trapped in the North Dakota tests using the (E,E) isomer with no apparent inhibition from the (Z,E) isomer.

Plans: Sufficient synthetic material will be purified (the majority has been synthesized) to carry out the field control experiment for the next two emergence seasons. In addition, tests will be conducted in an attempt to extend the field life of the attractant and to synergize the activity of the attractant. Efforts will be continued to establish the identity of the natural pheromone as (Z,E)-3,5-tetradecadien-1-ol acetate. More efficient synthetic routes to the two active isomers will be investigated.

1/ Forest Service Insect Research Laboratory, P. O. Box 227, Stoneville, Ms. 38776. 2/ New York Agricultural Experiment Station, Geneva, N.Y. 14456. 3/ Organic Chemical Synthesis Laboratory, Agricultural Environmental Quality Institute, Beltsville, Md. 4/ Division of Forest Insect & Disease Research, Forest Service, Washington, D.C. 5/ Shelterbelt Laboratory, Bottineau, N.D. 58318.



A Pheromone of the Tobacco Budworm,  
Heliothis virescens

J. H. Turlinson, D. Hendricks<sup>1/</sup>, R. E. Doolittle, E. R. Mitchell and  
M. M. Brennan

(Continuation of report 20 73(1-6))

Objectives: Isolation, identification, and synthesis of the pheromone produced by the female tobacco budworm, and incorporation of this pheromone into survey and/or control programs for this pest.

Methods: Virgin laboratory reared females, held on a reverse photoperiod, are collected when they are in the "calling" position. The whole female is rinsed with ether, the ether rinse is concentrated by evaporation, and the concentrate is chromatographed on Poragel 60A (37-75  $\mu$ ) eluted with hexane. The active fractions from this column are further purified by GLC on OV-1, Carbowax 20M, and Hi Eff IBP columns. All steps in the isolation are monitored by field cage bioassays. A sample of each fraction (about 50 FE in 0.5 ml solvent) is poured onto a piece of filter paper suspended in the middle of a cylindrical electric trap. Seven of these traps are placed in a cage containing hundreds to thousands of released, laboratory reared males. The traps are baited at night to coincide with the insects' activity period.

Results: The sex pheromone of the tobacco budworm has been identified as consisting of a mixture of (Z)-9-tetradecenal and (Z)-11-hexadecenal. The two compounds as well as the two corresponding (E) isomers were synthesized, mixed in the naturally occurring ratio and field tested. The synthesized compounds were equal in activity to live virgin females in field tests.

Plans: The two pheromone components are being produced commercially for formulation and field testing. A manuscript describing isolation, identification, and synthesis has been submitted to the Journal of Chemical Ecology.

<sup>1/</sup> Research Entomologist, Cotton Insects Research Laboratory, Brownsville, TX.



Isolation and Identification of the Sex Pheromone  
of the White Peachtree Scale

R. R. Heath, J. R. McLaughlin, J. H. Tumlinson, T. R. Ashley,  
and D. L. Chambers

Objective: As part of a program to develop an integrated pest management system for peach insects, the sex pheromone produced by the female white peach scale, Pseudaulacaspis pentagona, will be isolated, identified, synthesized, field tested, and incorporated into survey and control programs.

Methods: Live female scale are placed in an all glass vessel on potatoes, air passed over them, and volatiles are trapped from the air by cryogenic or absorption techniques. After extraction of the active material initial purification is done on a high pressure, high resolution liquid chromatography system using a UV detector. Further purification is done on GLC on OV-1 and Carbowax 20 M. All steps in the isolation are monitored by laboratory bioassay.

Results: Bioassay results indicate that the efficiency of the adsorption trapping was comparable to that of the cryogenic trapping. Because the adsorption trapping does not require constant attention it will be used for the collection of the pheromone. Activity is observable with as little as  $1 \times 10^{-3}$  female hours (FeHr) of trapping time. The pheromone has been partially purified by chromatography.

Plans: When 2 to 10  $\mu$ g of pure pheromone has been obtained, spectroscopic and other micro analytical data will be obtained for identification. Synthetic material will be prepared and compared for attractiveness.



### White Peach Scale Behavior

J. R. McLaughlin, T. R. Ashley, R. R. Heath, and D. L. Chambers

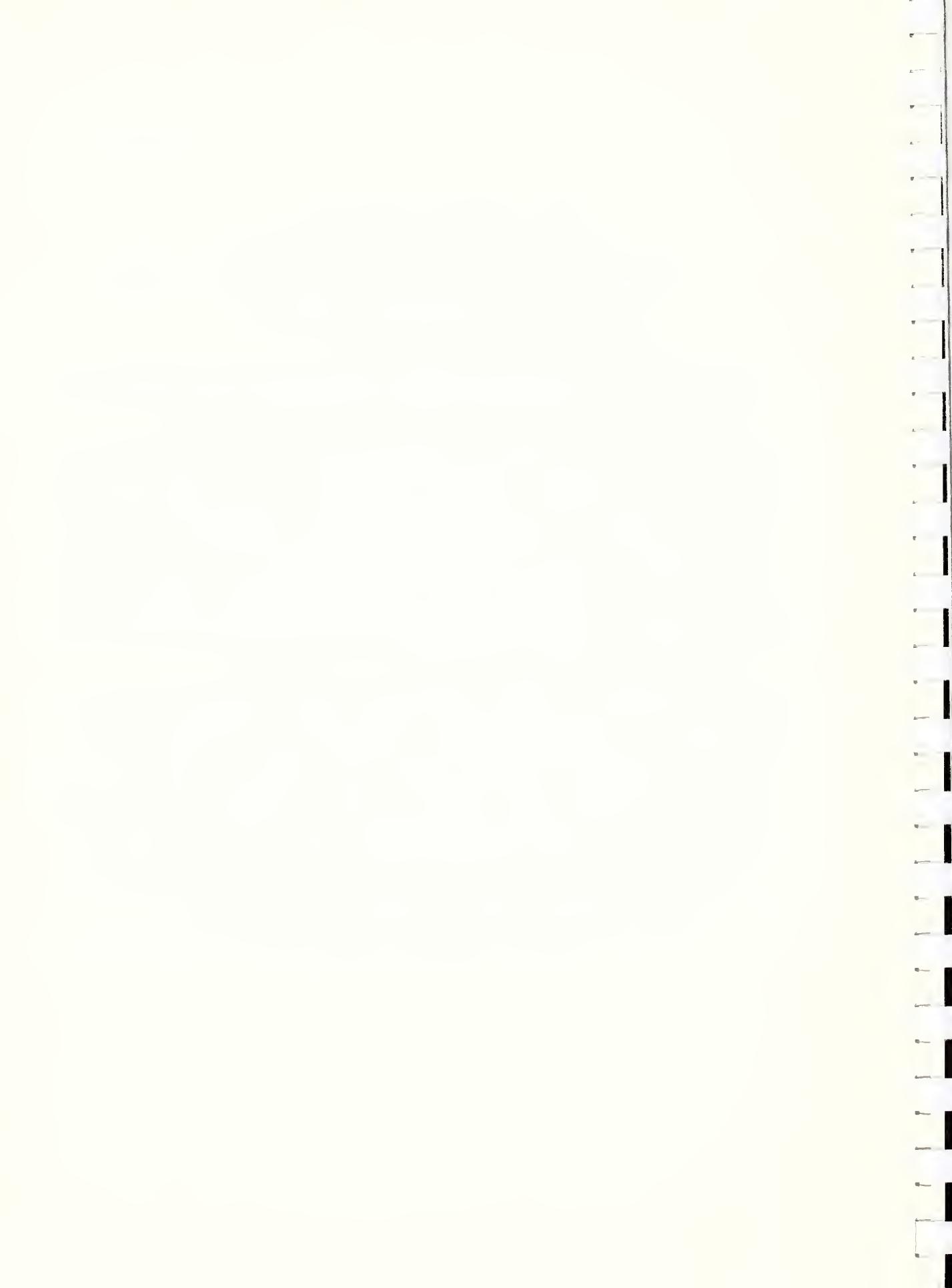
(Continuation of report 24 73(7-12))

Objective: To describe pre-mating and mating behavior of the white peach scale, Pseudaulacaspis pontagona (Targ.), with emphasis on the role of pheromones.

Methods: A laboratory bioassay in petri dish arenas has been developed by McLaughlin and Chambers to monitor and quantify the response of males to volatiles removed from air blown over sexually active females. Ashley has developed a technique for monitoring the daily emergence pattern of males in the laboratory, and McLaughlin is developing techniques for trapping males in the field using live females and fractions of trapped volatiles as bait. Heath has developed a technique that will facilitate the determination of the rhythms of pheromone release by the females in the laboratory. Field and laboratory observational studies will further elucidate the pre-mating and mating behavior of the species.

Results: The laboratory bioassay is sensitive to ca.  $10^{-3}$  female hours of liquid chromatographed fractions of air-borne volatiles from virgin females; however, the type of response monitored, very short range (2-3 cm) walking orientation and copulatory stimulation, must be augmented with lab and field investigation of longer range flight response. Males on a 14:10, L:D, light cycle emerge only during the period from 6-11 hrs after lights on. The cue(s) mediating this behavior is yet to be determined. Field trapping with virgin females has had limited success and we are still attempting to determine when females are most attractive and the biological significance of the pheromone.

Plans: This is a continuing long range project toward the stated objective.



Borer Pheromones (Family Sesiidae)

J. H. Tumlinson et al.<sup>1/</sup>

(Expansion and continuation of report 19 73(7-12))

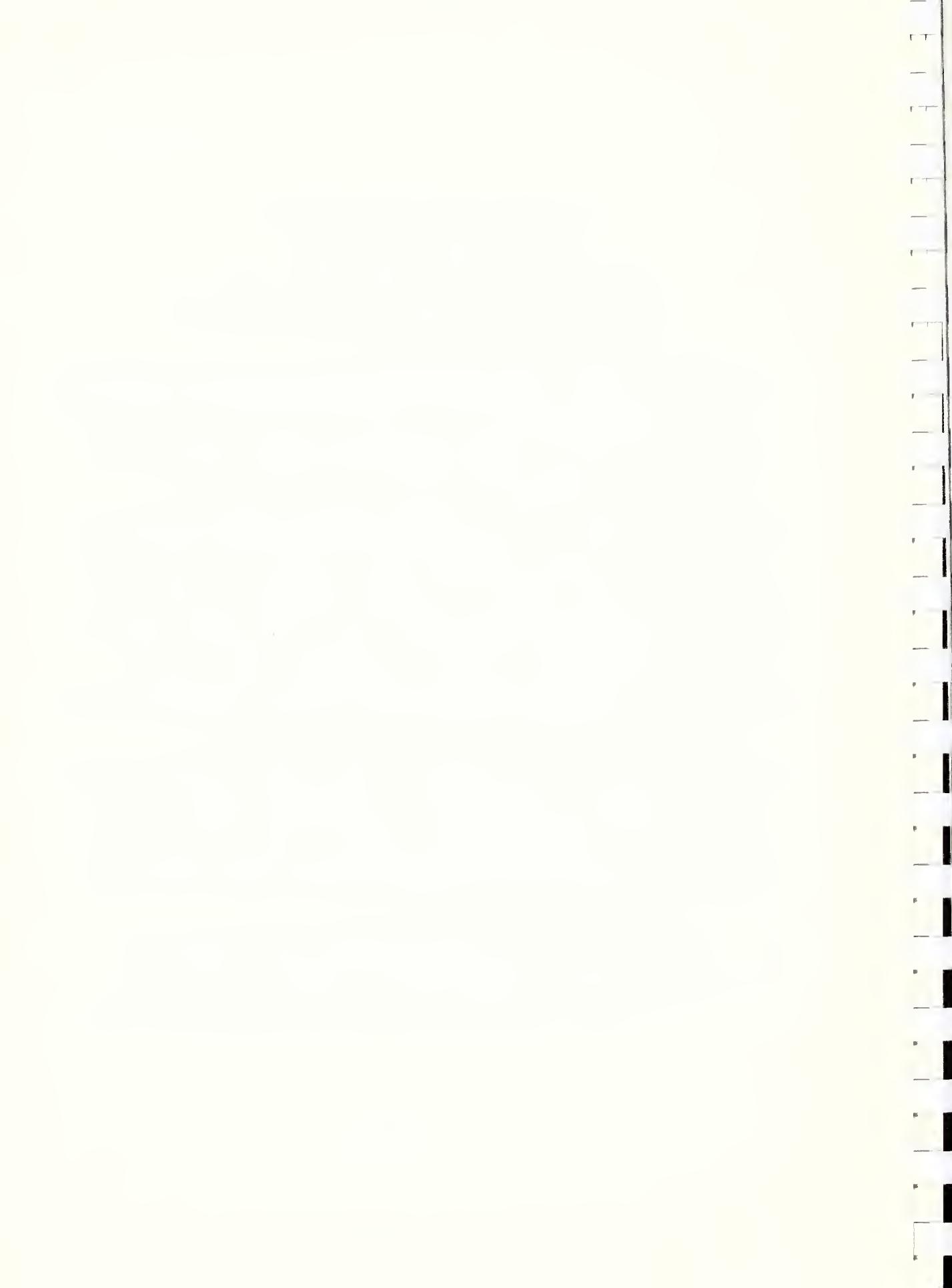
Objectives: As part of a program to develop an integrated pest management system for peach insects, the sex pheromones of the lesser peachtree borer and peachtree borer were isolated, identified, and synthesized and will be incorporated into survey and control programs for these insects. Additionally, these pheromones and isomeric compounds will be tested for survey and control of other sesiid species.

Methods: The isolation, identification, synthesis, and bioassay of the lesser peachtree borer and peachtree borer pheromones are described by Yonce et al. (1974) and Tumlinson et al. (1974). The (E,Z)-(Z,E)- and (Z,Z)- geometrical isomers of 3,13-octadecadien-1-ol acetate (ODDA) have been synthesized and purified in quantities large enough for this seasons field tests by high pressure liquid chromatography on silver nitrate treated silica gel. Various field tests are being conducted by the many entomologists cooperating in this study.<sup>1/</sup> These include formulation and trapping experiments, release-recapture, dispersal, communication, disruption, and survey and trapping of other Sesiidae species.

Results: It is not within the scope of this report to list the results of all the cooperating entomologists.<sup>1/</sup> The E,Z- and Z,Z-isomers have been thoroughly tested in the field and have performed very well in all tests although considerable work is still needed in formulation to get optimum results over long periods of time. Several other species of sesiids have been captured in traps baited with Z,Z- and E,Z-ODDA and mixtures of the two.

Plans: We will continue to supply pheromones to all cooperating entomologists throughout this season for the various field tests. Pheromone for next season is being prepared commercially and we are working closely with the manufacturer to insure high quality material.

<sup>1/</sup> See following page for list of cooperators.



Cooperators:AddressChemists:

R. E. Doolittle  
 R. R. Heath  
 A. T. Proveaux

Insect Attractants and Basic Biology Lab.  
 " " " " " "

Entomologists

J. R. McLaughlin  
 E. R. Mitchell  
 C. R. Gentry  
 C. E. Yonce  
 R. E. Dolphin  
 W. D. Duckworth  
 T. D. Eichlin  
 R. L. Holloway  
 M. G. Karandinos  
 D. G. Nielsen

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 Fruit and Tree Nuts Laboratory, Byron, Ga.  
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 Fruit Insect Res. Invest., Vincennes, Ind.  
 Smithsonian Institute, Wash. D.C.  
 Div. Plant Industry, Sacramento, Calif.  
 Dept. of Ent. Clemson Univ., Clemson S.C.  
 Dept. of Ent. Univ. of Wis. Madison, Wis.  
 Ent. Dept. Agr. Res. & Dev. Ctr. Wooster,  
 Ohio



Dispensing Sesiidae Sex Pheromones in the Field

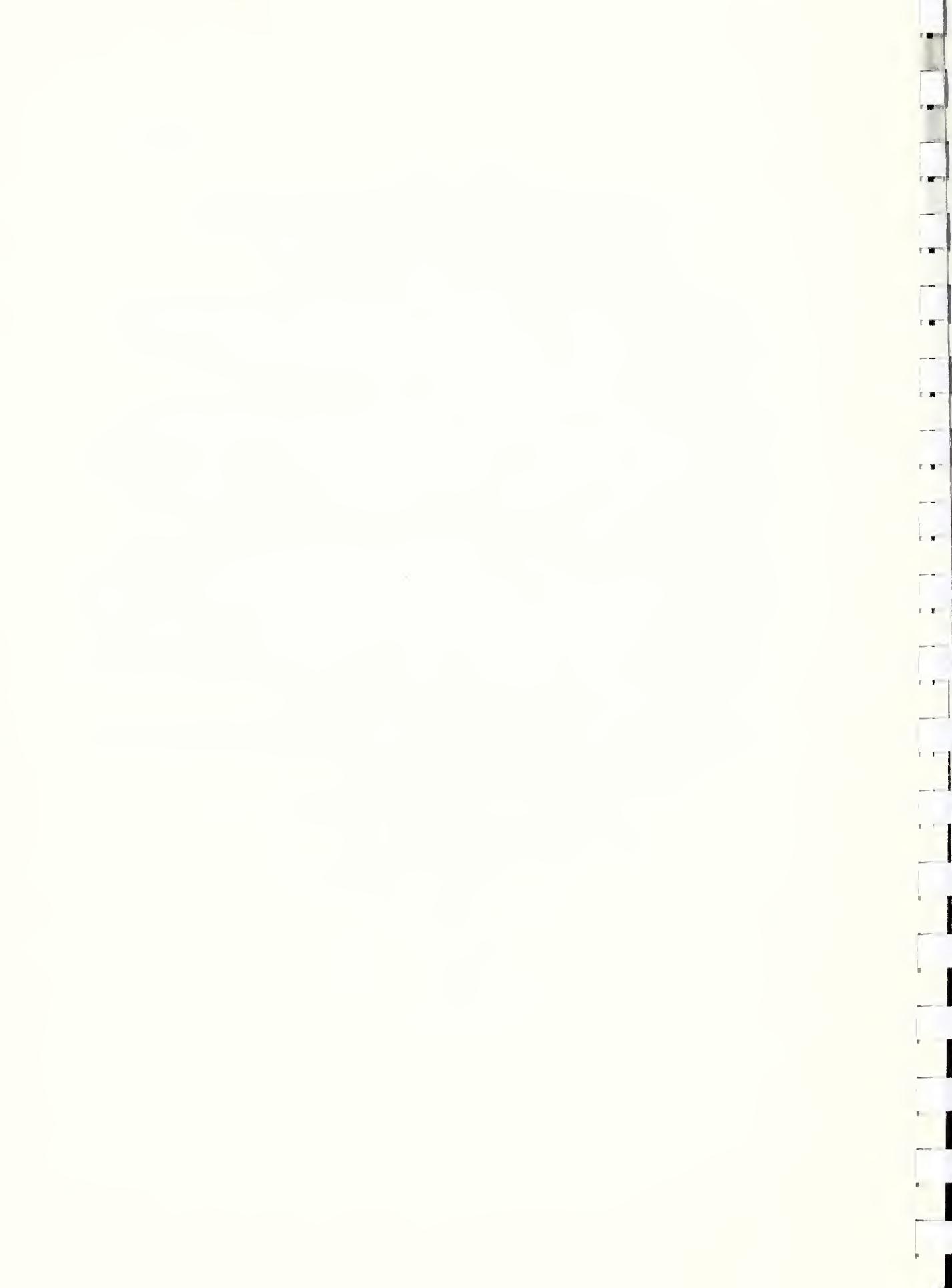
J. R. McLaughlin and J. E. Brogdon

Objectives: To develop methods for efficiently dispensing the pheromones (3,13 octadecyldienyl acetates) and protecting them from the rapid degradation to which they are subject.

Methods: We dispense the pheromones of the lesser and major peachtree borer as liquid films evaporated from stainless steel surfaces. We have tested 2 phenylenediamine antiozonants (Union Oil Products Co. Nos. 88 and 688), the UOP product, Sustain 6 (BHA and BHT plus vegetable oil), and trioctanoin, (the keeper used with gypsy moth pheromone).

Results: UOP 88 and Sustain 6 both greatly extended the field life of the pheromones. Tricotanoin was not effective in our test; however, we may need to reevaluate its effect on evaporation rate. UOP 688 at a volumetric ratio of ca. 1 (pheromone) to 4,000 (antiozonant) extended from 1 to 2 days the attractive life of 10 ng of the borer pheromones to ca. 3 weeks. Dilute mixtures of UOP 688 and 88 were not effective.

Plans: Major emphasis will be on quantification of our dispensers and refinement of technique.



Exploitation of the Sex Pheromone Behavior  
of Sesiidae: Disruption of Mating Communication

J. R. McLaughlin, R. E. Doolittle, E. R. Mitchell, J. H. Tumlinson,  
C. R. Yonce,<sup>1/</sup> and R. Gentry<sup>1/</sup>

Objective: To assess the feasibility of impairing the mating process in the lesser peachtree borer and peachtree borer by permeating their surrounding atmosphere with synthetic sex pheromones and related compounds.

Methods: Preliminary experiments were conducted in which either the lesser peachtree borer (LPTB) sex pheromone, (E,Z)-3,13-octadecadienyl acetate (E,Z-ODDA) or the peachtree borer pheromone, Z,Z-ODDA, was evaporated into the atmosphere surrounding traps baited with virgin LPTB females. The effect of such treatments was assessed relative to the capture of males by females in a nearby untreated area of the peach orchard. The treatment was alternated daily between the two trapping sites.

Results: The capture of males by females in pheromone-permeated atmospheres was reduced relative to the control. Complete interruption of communication was achieved with approximate levels of both isomers of ODDA and at about the same concentration of each isomer. Further evaluation and quantification is in progress.

Plans: Laboratory bioassay will more fully elucidate the effects of E,Z and Z,Z ODDA on male LPTB. This fall, field tests of a similar nature will be conducted with the major peachtree borer.

<sup>1/</sup> Research Entomologists, Southeastern Fruit & Tree Nut Research Station Laboratory, P.O. Box 87, Byron, Ga.



Exploitation of the Sex Pheromone Behavior of Sesiidae:  
Trapping and Male Annihilation Techniques

J. R. McLaughlin, E. R. Mitchell, J. E. Brogdon,  
C. R. Yonce,<sup>1/</sup> and R. Gentry<sup>1/</sup>

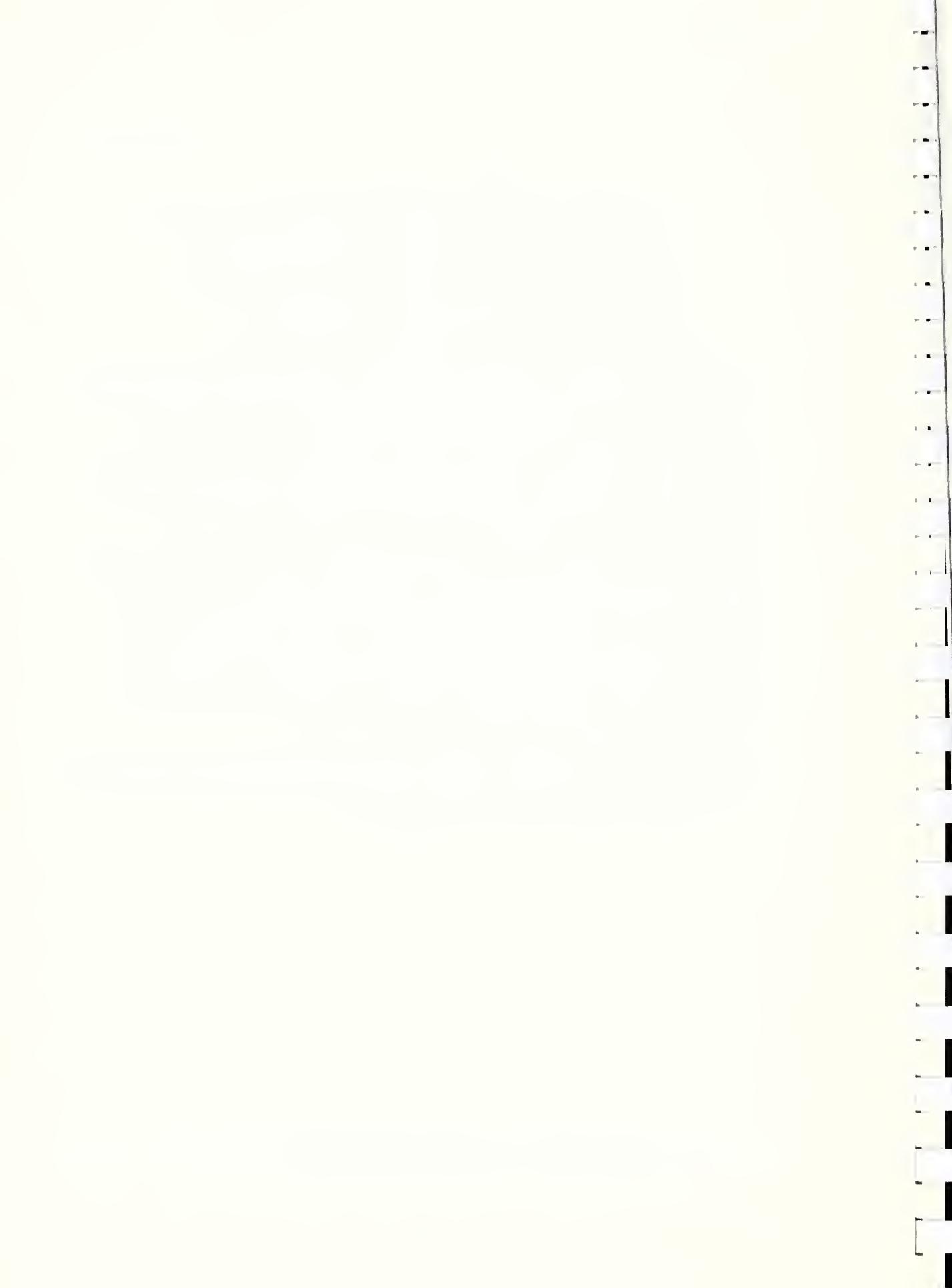
Objective: To assess the feasibility of impairing the mating process by trapping or otherwise annihilating male lesser peachtree borers or peachtree borers before they can mate with females.

Methods: We have assessed the effect of trap location and color on the capture of male lesser borers in peach orchards. The Pherocon-1C or similar sticky traps were used. These were baited with synthetic sex pheromone.

Results: Traps placed at the top of peach trees captured significantly more males than traps placed at the outer-middle part of the tree canopy. Traps placed in the low canopy captured the fewest moths. No significant differences were observed among captures by traps placed in the NW, NE, SW, or SE quadrants of trees. A wide range of colors from blues through reds and including black and white did not affect the capture of males by the pheromone-baited traps.

Plans: Our major emphasis will be on the development of techniques for the annihilation of males lured with sex pheromone to toxicant-treated sites.

<sup>1/</sup> Research Entomologists, Southeastern Fruit and Tree Nut Research Station Laboratory, P.O. Box 87, Byron, Ga.



Control of Stored-Product Lepidoptera  
With Sex Pheromone

L. L. Sower, K. W. Vick and J. A. Coffelt

(Continuation of report 27 73(7-12))

Objectives: To make sound recommendations as to whether or not certain lepidopterous pests of stored products can be directly controlled by introducing synthetic sex pheromone into their environment. The immediate objectives are to determine the effects of pheromone concentrations and population densities on the mating frequencies and behavior of Indian meal moths.

Methods: Tests were conducted in 0.2 to 90 m<sup>3</sup> enclosed environments in the presence of several quantities of (Z,E)-9,12-tetradecadien-1-ol acetate. Mating frequencies were monitored at several population densities.

Results: A dose/response curve at constant population density was previously reported. The population density/mating frequencies relationship was established (Fig. 1). The synthetic pheromone markedly reduced mating frequencies at low population densities; however, the use of synthetic pheromone does not appear promising for suppression of Indian meal moths in stored commodities where high population densities already occur.

The release of ozone into enclosed environments destroyed the attractiveness of sex pheromone vapors evaporating from synthetic sources or from calling females. These results suggest a method for removing unwanted pheromone residues and vapors from experimental apparatus (in cooperation with D. L. Silhacek).

Plans: Similar studies will be initiated with the Angoumois grain moth.

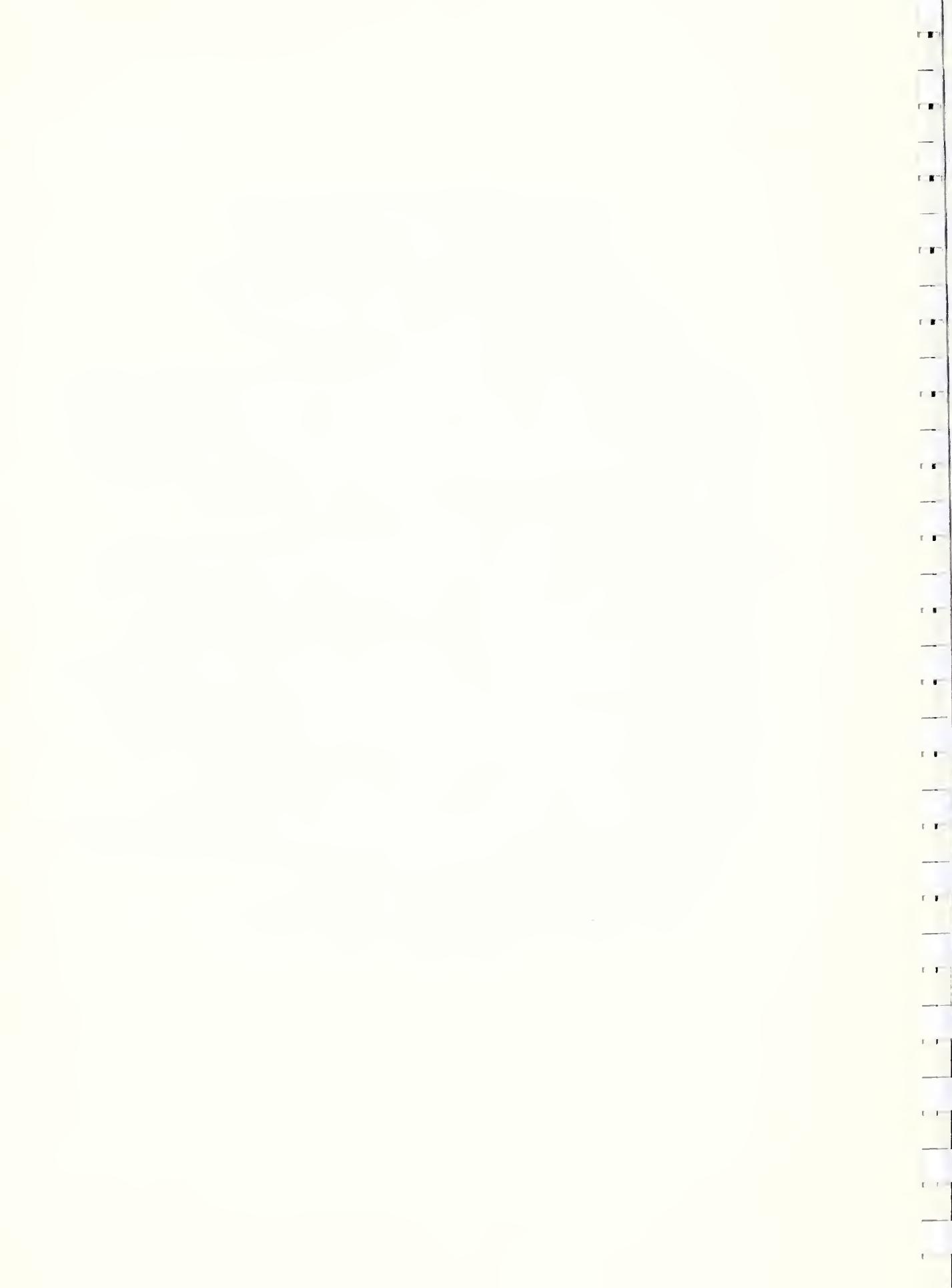
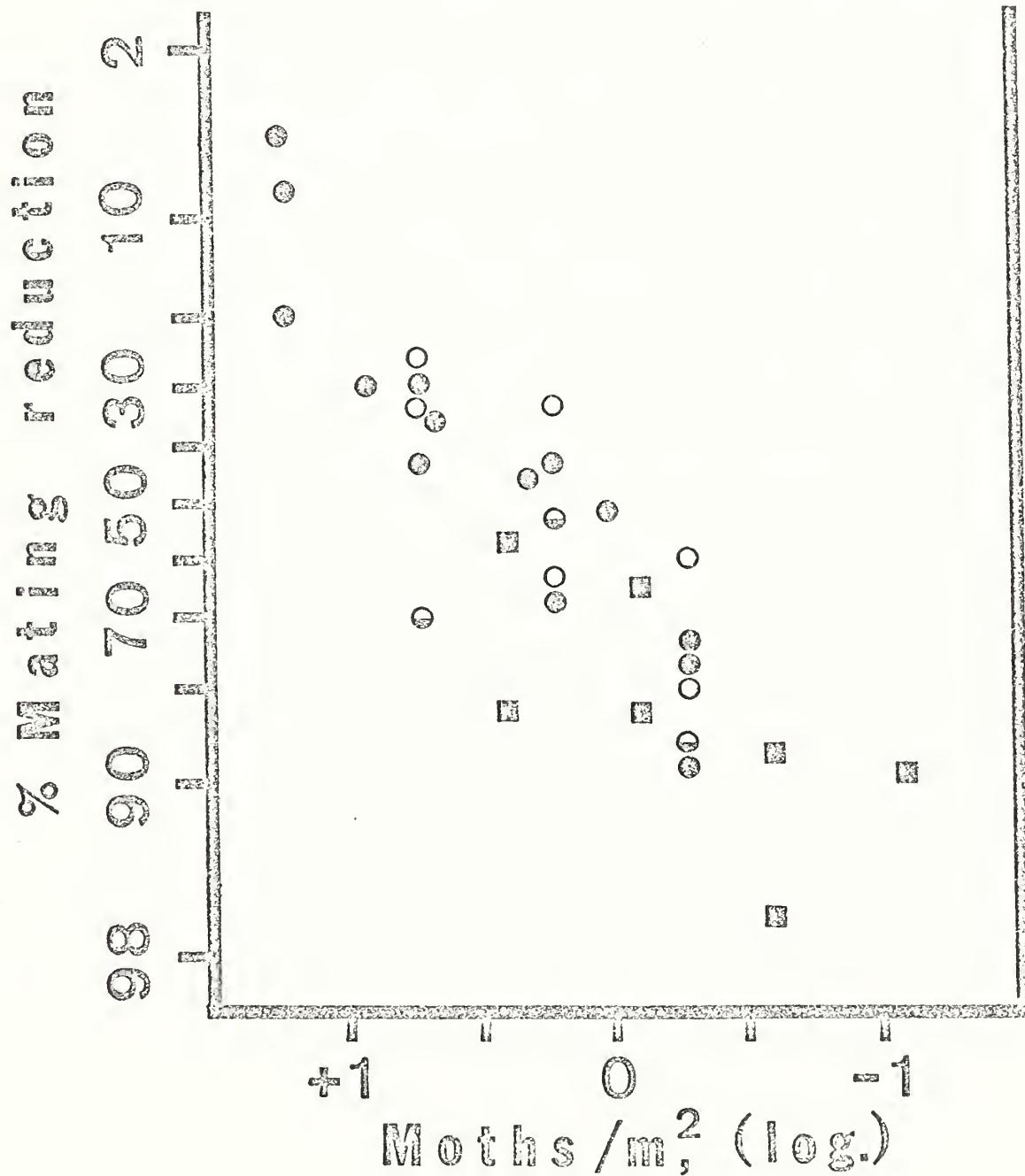
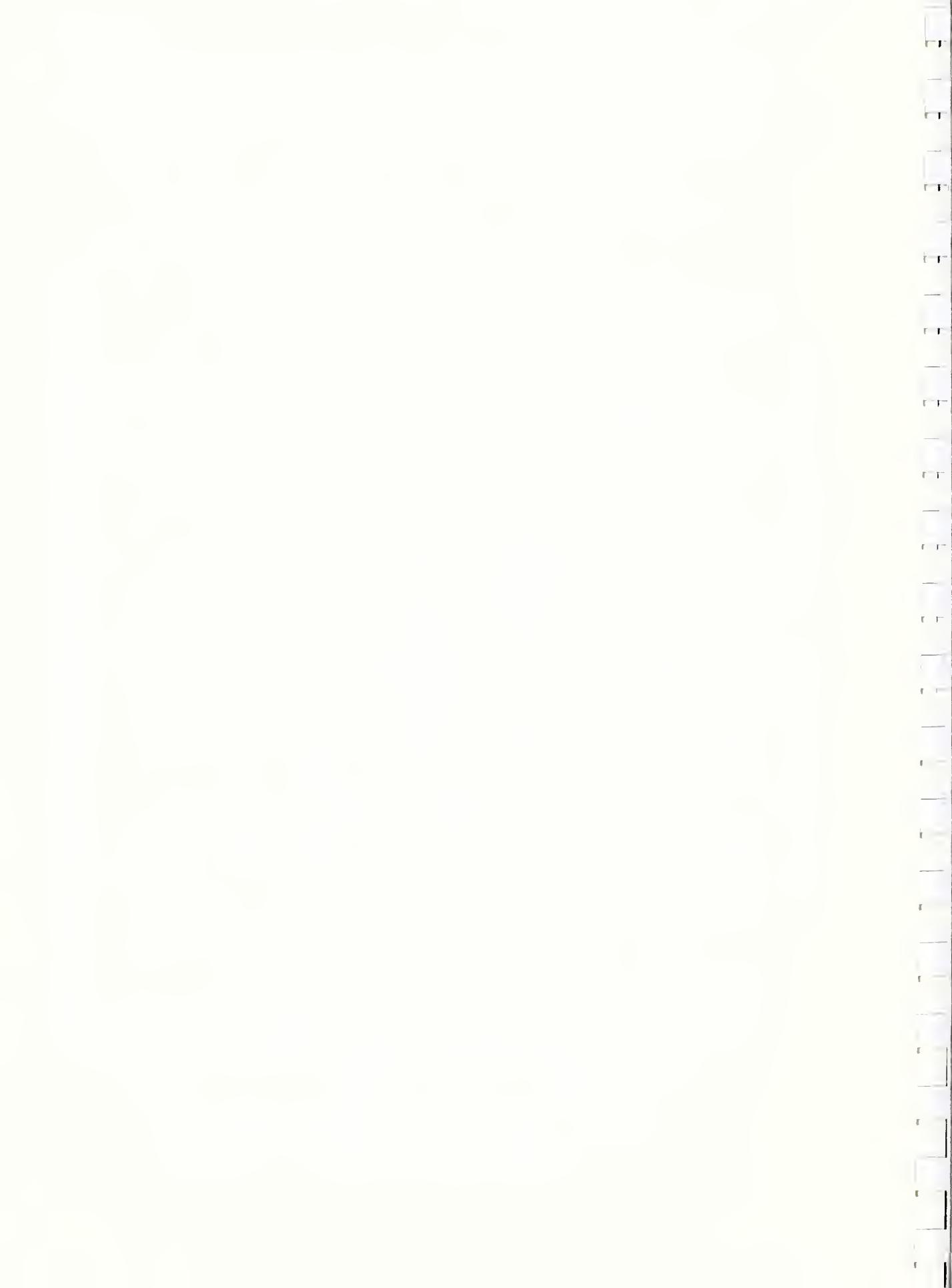


Fig. 1. Reduction of mating each night, relative to control tests, of Indian meal moths at several population densities. The several symbols indicate different pheromone dosages. Control data were taken in the absence of synthetic pheromone.





Resistance to Atmospheric Permeation as a  
Means for Mating Control of Lepidoptera

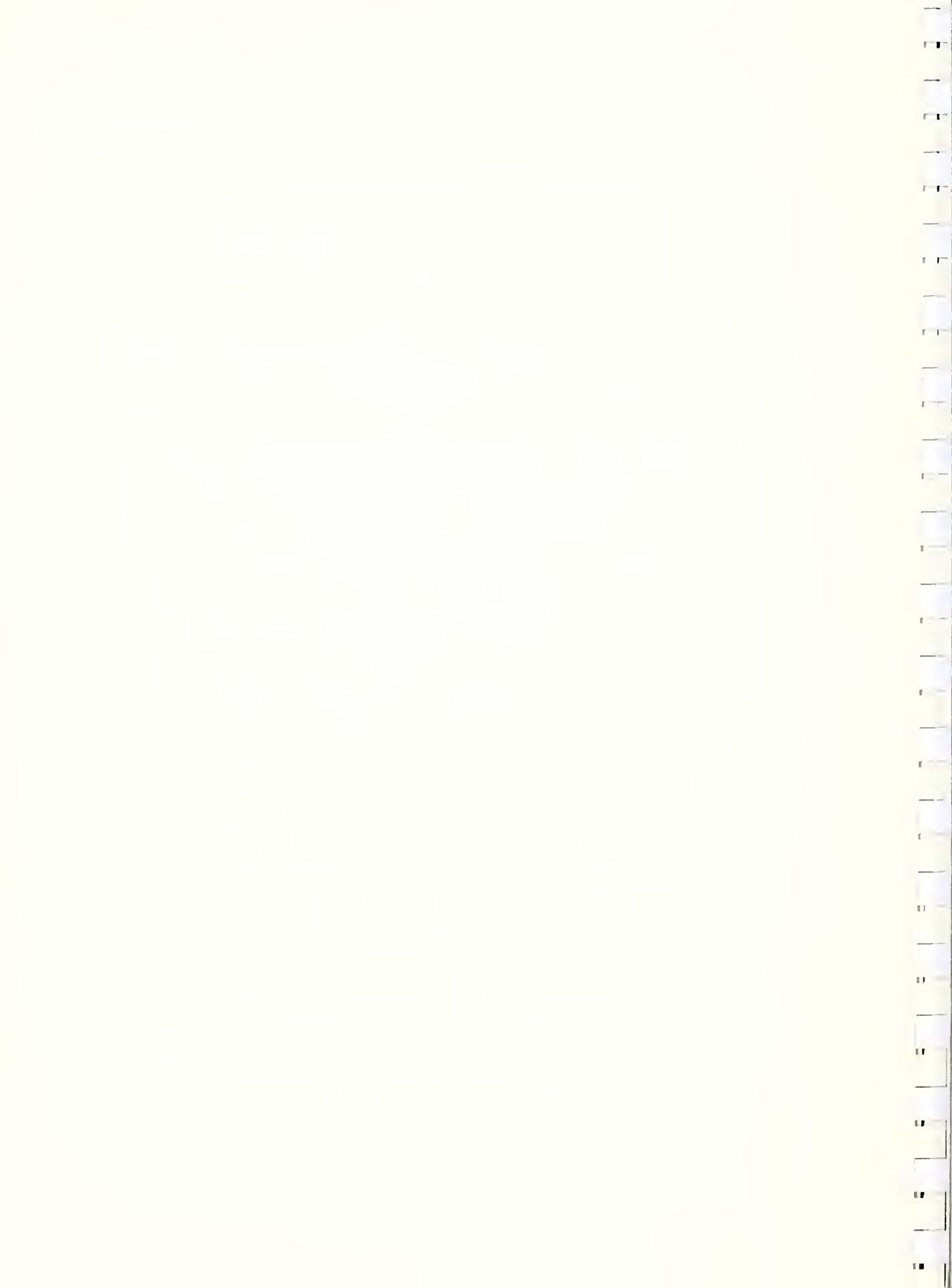
J. R. McLaughlin and L. L. Sower

Objective: To determine the potential selective influence of a pheromone-permeated atmosphere on the premating behavior of a moth.

Method: Adults from sub-populations taken from a laboratory colony of the Indian meal moth are mated in standardized cages. Each generation of some lines must mate in a sex pheromone-permeated atmosphere while other lines are allowed to mate with no interference. After several generations these lines will be tested for various premating parameters and compared with the parent colony.

Results: No effects are apparent after 3 generations. However, definitive tests have not been conducted.

Plans: We will pursue the objective as stated. Other species may be investigated at some future date.



Use of Sex Pheromones for Behavioral Control  
of Loopers and Related Noctuid Species

J. R. McLaughlin, E. R. Mitchell and D. L. Chambers

(Continuation of report 25 73(1-6))

Objectives: This study explores methods for controlling population levels by manipulating the sex pheromone communication systems of the cabbage looper, soybean looper, and several related species. The technique whereby the sex pheromone is continually evaporated into the air over a test area at a concentration above the male behavioral threshold (environmental permeation) is being examined. To test this technique adequately against wide-ranging species such as the cabbage and soybean loopers, large land areas must be treated. We are developing a system for broadcasting the pheromone over such areas.

Methods: Micro-encapsulated cabbage looper pheromone (NCR process-- pheromone as 2.2% in xylene, 25 to 125 micron gel capsules, 25% plastic treatment) was formulated with hydroxyethyl cellulose and a latex sticker as suggested by Dr. Morton Beroza, Organic Chemical Synthesis Laboratory, ARS, USDA, Beltsville, Md. Ground application of formulations delivered the pheromone at from 1.25 to 10 g/ha to 30 x 30 m plots in cabbage fields using ULV and conventional spray equipment. A pheromone-baited trap was placed at the center of each plot and treatments were replicated 1 to 3 times.

Results: In no instance did treatment reduce captures of male cabbage loopers in the pheromone-baited traps relative to the untreated controls. We are presently assessing the pheromone-release characteristics of the microcapsules.

Plans: We plan to continue testing various proposed methods for dispensing the pheromone. Immediate emphasis will be on upgrading our techniques for measuring release rates. Micro-encapsulation will be investigated more fully.



Cabbage Looper: Inhibition of Pheromone Perception  
by Males with Synthetic Acetates

E. R. Mitchell

Objective: To evaluate the effects of synthetic acetates on sex pheromone communication in the cabbage looper (CL).

Methods: Experiment 1.--This study was conducted April 5-12 at Hastings, Fla., to determine whether 25  $\mu$ l/vial of  $\underline{Z}\text{-7-dodecen-1-ol acetate}$  ( $\underline{Z}\text{-7-dda}$ ) would attract CL males when the same electric grid trap was baited simultaneously with this compound and with a separate vial containing 25  $\mu$ l of  $\underline{Z}\text{-9-dodecen-1-ol acetate}$  ( $\underline{Z}\text{-9-dda}$ ),  $\underline{Z}\text{-9-tetradecen-1-ol acetate}$  ( $\underline{Z}\text{-9-tda}$ ), or ( $\underline{Z},\underline{E}\text{-9,12-tetradecadien-1-ol acetate}$ ) ( $\underline{[Z,E]-9,12-tda}$ ). Captured insects were collected every 2-3 days, and the treatments were rotated one position after each collection. Each treatment was replicated 10 times (2 replicates/treatment x 5 collections).

Experiment 2.--The disruptive effect of  $\underline{Z}\text{-9-dda}$ ,  $\underline{Z}\text{-9-tda}$ , and ( $\underline{Z},\underline{E}\text{-9,12-tda}$ ) on pheromone communication in the CL also was assessed by determining whether males could locate a pheromone source ( $\underline{Z}\text{-7-dda}$ ) in small field plots when they were simultaneously exposed to a chemical-impregnated atmosphere. Four experimental areas (ca. 150 m apart) were established in a cabbage field at Hastings, Fla.  $\underline{Z}\text{-9-dda}$ ,  $\underline{Z}\text{-9-tda}$ , or ( $\underline{Z},\underline{E}\text{-9,12-tda}$ ) was evaporated into the air from 16 dispensers (1.25 ml polyethylene vials each containing 25  $\mu$ l chemical) arrayed at 3-m intervals in a 4 x 4 checkerboard pattern. A can trap baited with 0.1 ml  $\underline{Z}\text{-7-dda}$  was positioned in the center of each plot. Captured insects were collected every 2-3 days (April 17-22), and the 3 treatments and a control were rotated one position after each collection. Each of the 3 collections was considered a replicate.

Results:  $\underline{Z}\text{-9-dda}$ ,  $\underline{Z}\text{-9-tda}$ , and ( $\underline{Z},\underline{E}\text{-9,12-tda}$ ) significantly reduced captures of CL males when dispensed from the same trap as the CL sex pheromone,  $\underline{Z}\text{-7-dda}$  (Table 1). However, none of these chemicals had any significant influence on the capture of CL males when they were evaporated into the atmosphere surrounding pheromone-baited traps (Table 2).

Plans: The factors responsible for inhibition of CL males in the 1st test will be further elucidated in the laboratory.



Table 1. Captures of male cabbage looper moths in electric grid traps baited with  $\underline{Z-7}$ -dda (25  $\mu$ l) plus 25  $\mu$ l of  $(\underline{Z},\underline{E})9,12$ -tda,  $\underline{Z-9}$ -dda or  $\underline{Z-9}$ -tda. Hastings, Fla., April 5-12, 1974.

Bait	Mean no. ♂ captured/ trap /night <sup>a</sup> /
25 $\mu$ l $\underline{Z-7}$ -dda	54.4 a
25 $\mu$ l $\underline{Z-7}$ -dda + 25 $\mu$ l $(\underline{Z},\underline{E})9,12$ -tda	10.3 b
$\underline{Z-9}$ -dda	4.9 b
$\underline{Z-9}$ -tda	1.7 b

a/ Means with letters in common are homogenous ( $P = 0.05$ , Duncan's multiple range test).

Table 2. Captures of cabbage looper males in pheromone-baited can traps placed within grids of chemical evaporators. Hastings, Fla., April 17-22, 1974.

Chemical	Control Plot	Treated Plot
$(\underline{Z},\underline{E})9,12$ ,tda	28.6	17.4
$\underline{Z-9}$ -dda	28.6	23.7
$\underline{Z-9}$ -tda	28.6	45.8

a/ Differences among means in the control and treated plots were not significant ( $P = 0.05$ , Student's  $t$ -test).



Beet Armyworm: Inhibition of Pheromone Perception by Males with Synthetic Acetates

E. R. Mitchell

Objective: To evaluate the effects of synthetic acetates on sex pheromone communication in the beet armyworm (BAW).

Methods: Experiment 1.--This experiment was conducted March 22-April 3 at Hastings, Fla., to determine if virgin female BAW would attract male BAW when the same electric grid trap was baited simultaneously with females (3) and with a 1.25 ml polyethylene vial containing 25  $\mu$ l of Z-7-dodecen-1-ol acetate (Z-7-dda), Z-9-dodecen-1-ol acetate (Z-9-dda) or Z-9-tetradecen-1-ol acetate (Z-9-tda). Captured insects were collected every 2-3 days, and the treatments were rotated one position after each collection. Each treatment was replicated 10 times (2 replicates/treatment X 5 collections).

Experiment 2.--The disruptive effect of Z-9-dda, Z-9-tda, and (Z,E)-9,12-tetradecadien-1-ol acetate ([Z,E]-9,12-tda) on pheromone communication in the BAW was evaluated further by determining if males could locate calling females in small field plots when they were simultaneously exposed to a chemical-impregnated atmosphere. Four experimental areas (ca. 300 m apart) were established in and around cabbage fields at Hastings, Fla. The chemicals were evaporated into the air from 16 polyethylene vial dispensers (1.25 ml) each containing 25  $\mu$ l chemical. The vials were supported on wooden stakes and arrayed at 3-m intervals in a 4 x 4 checkerboard pattern. An electric grid trap baited with 3 virgin female BAW was positioned in the center of each plot. Captured insects were collected every 2-3 days, and the 3 treatments and the control were rotated 1 position after each collection. Each collection, therefore, was considered to be a different replicate.

Results: Each of the chemicals tested significantly reduced captures of BAW males when used in the same trap with BAW females (Table 1). Cabbage looper males were captured only in traps baited with Z-7-dda ( $X=72.2/\text{trap/night}$ ) and Z-7-dda plus BAW females ( $X=53.9/\text{trap/night}$ ). The difference in male captures among these 2 treatments was not significant.

Each of the 3 chemicals tested in the atmospheric permeation experiment (Table 2) was effective in disrupting pheromone communication in the BAW. A reported pheromone of this species, (Z,E)-9-12-tda, was particularly effective, reducing male captures by 96% over the corresponding control trap.

Plans: Continue to study the effects of various synthetic chemicals on the behavior of the BAW.

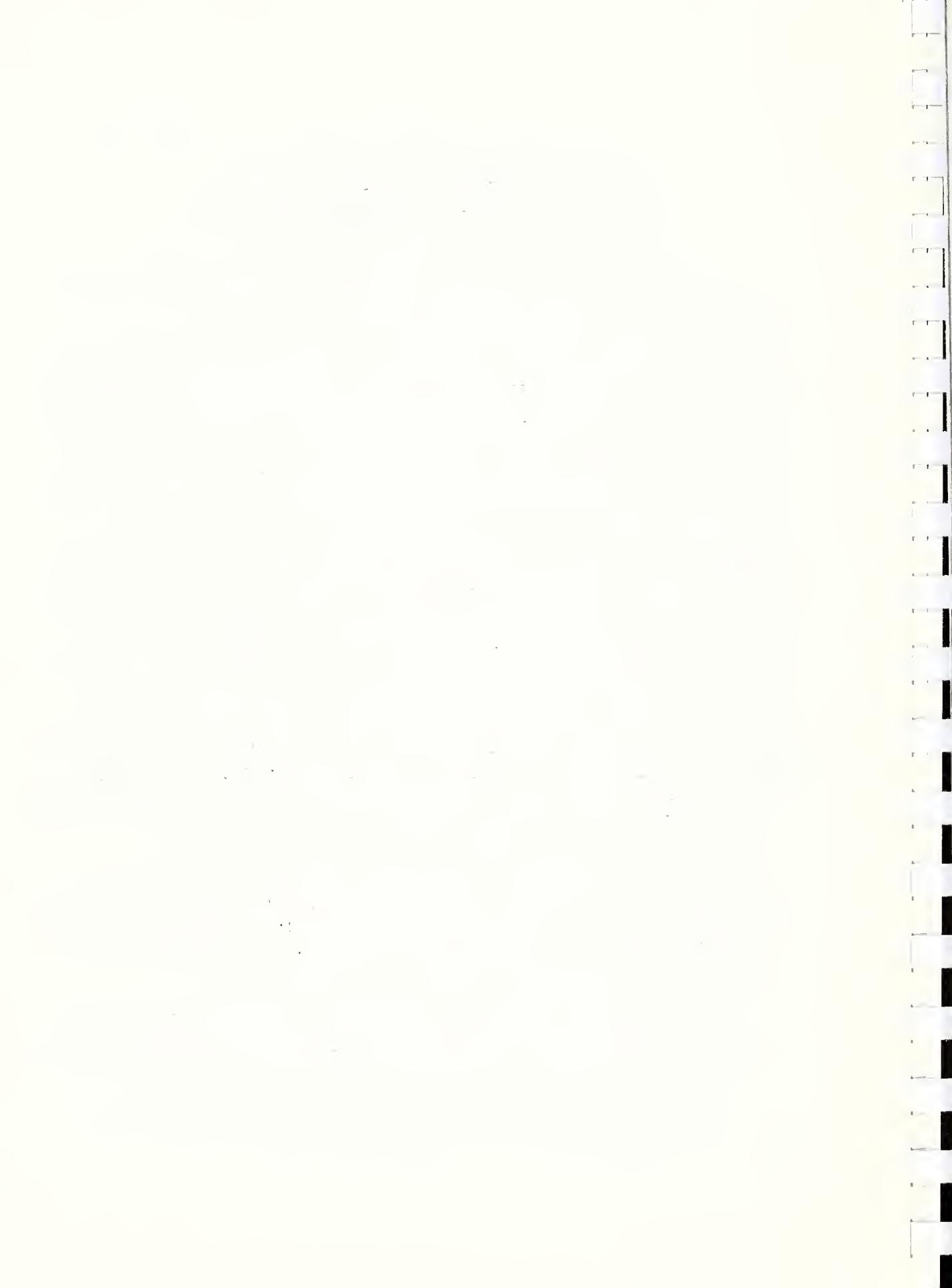


Table 1. Captures of male beet armyworm (BAW) moths in electric grid traps baited with virgin females. Hastings, Fla., 1974.

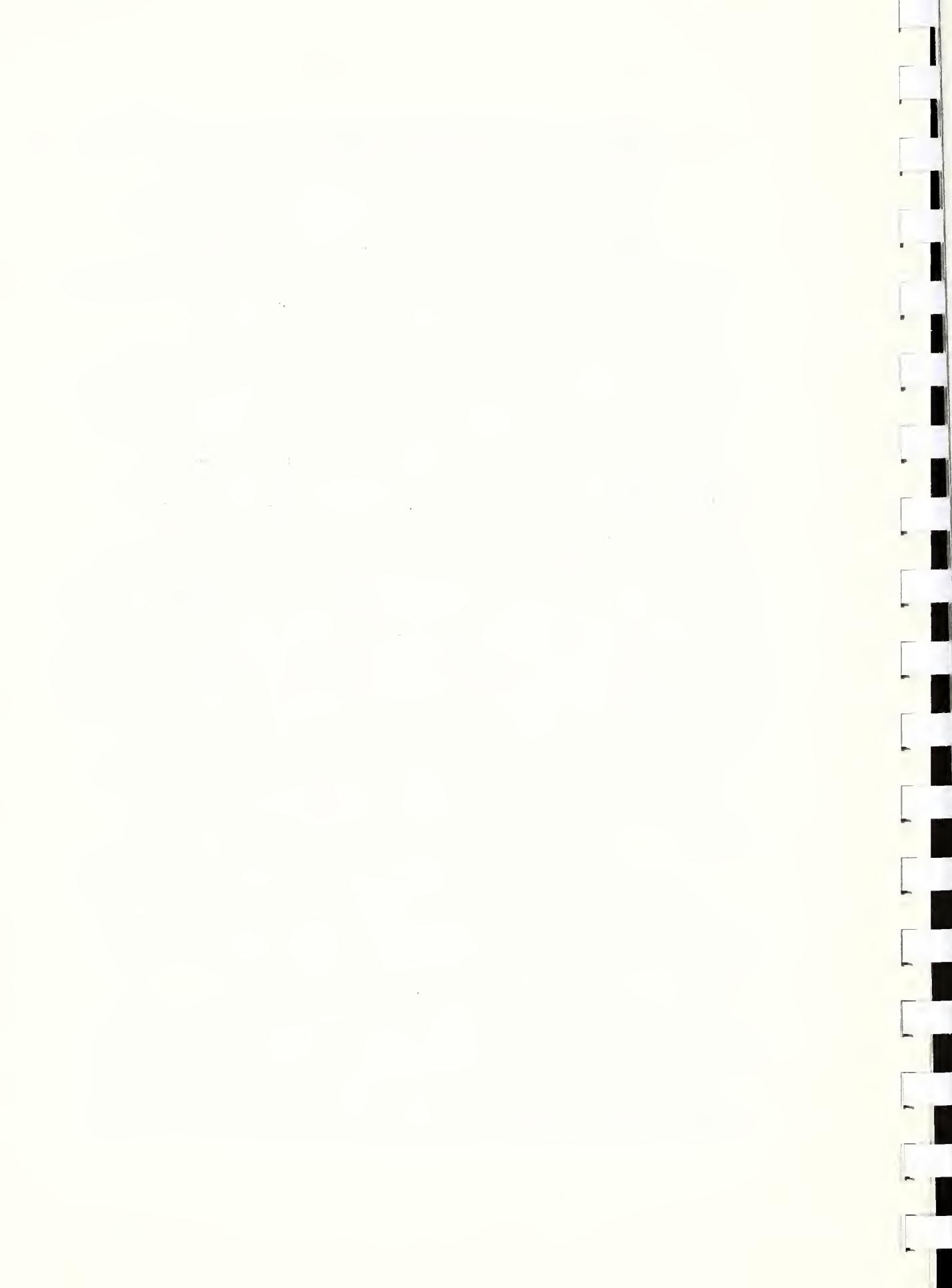
Bait	Mean no. ♂ captured/ trap/night <sup>a</sup>
3 BAW ♀♀	24.5 a
3 BAW ♀♀ + 25 µl	
<u>Z-7-dda</u>	11.2 b
<u>Z-9-dda</u>	1.9 c
<u>Z-9-tda</u>	0.7 c
25 mg <u>Z-7-dda</u>	.0 c
25 mg <u>Z-9 dda</u>	.0 c

a/ Means with letters in common are homogenous ( $P = 0.05$ , Duncan's multiple range test).

Table 2. Captures of beet armyworm males in ♀-baited electric grid traps placed within grids of chemical evaporators. Hastings, Fla., April 24-May 17, 1974.

Chemical	Replicates	Mean no. ♂ captured/ trap/night <sup>a</sup>		% reduction over control
		Control Plot	Treated Plot	
<u>Z-9-dda</u>	6	376.5 a	95.7 b	74.6
<u>Z-9-tda</u>	7	365.6 a	59.1 b	83.8
( <u>Z,E</u> )-9,12-tda	8	330.7 a	13.1 b	96.0

a/ Means within rows followed by different letters differ significantly ( $P=0.05$ , Student's t-test).



Acoustical Properties as a Means  
of Measuring Insect Quality

J. C. Webb, J. L. Sharp, D. L. Chambers, J. C. Benner  
and B. J. Smittle<sup>1/</sup>

(Continuation of report 35 73(7-12))

Objectives: To determine the changes that occur in the acoustical physiology of the Caribbean fruit fly, Anastrepha suspensa (Loew) after exposure to various treatments (such as gamma radiation, heat, diet changes, strain selection, etc.).

Methods: The flies are reared in the laboratory, and after they have received the appropriate treatment the flight and signalling sounds are recorded. Analyses of these sounds are made to determine the changes, if any, caused by the treatment.

Results: Flies irradiated with cobalt 60 in air 4-5 days before emergence were tested. The acoustical properties of the flight and signalling sounds were recorded. Two additional replications are to be run before the data are analyzed. Also, the acoustical properties of the signalling sound of male flies reared on different diets were recorded. Preliminary recordings have been made of males reared on bagasse, corncob, and agar based diets.

Plans: With these data and our ability to identify behavior patterns (flight, aggression, signalling, etc.) by analyzing sound production as background information, we will evaluate the use of sound analysis as a measure of quality. Flies selected through rearing, with various nutritional or rearing histories, or subjected to various treatments such as irradiation or marking will be compared through sound analysis and potential detrimental effects identified.

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The Signalling Sound of Anastrepha suspensa  
Males and its Apparent Significance to Pair Formation

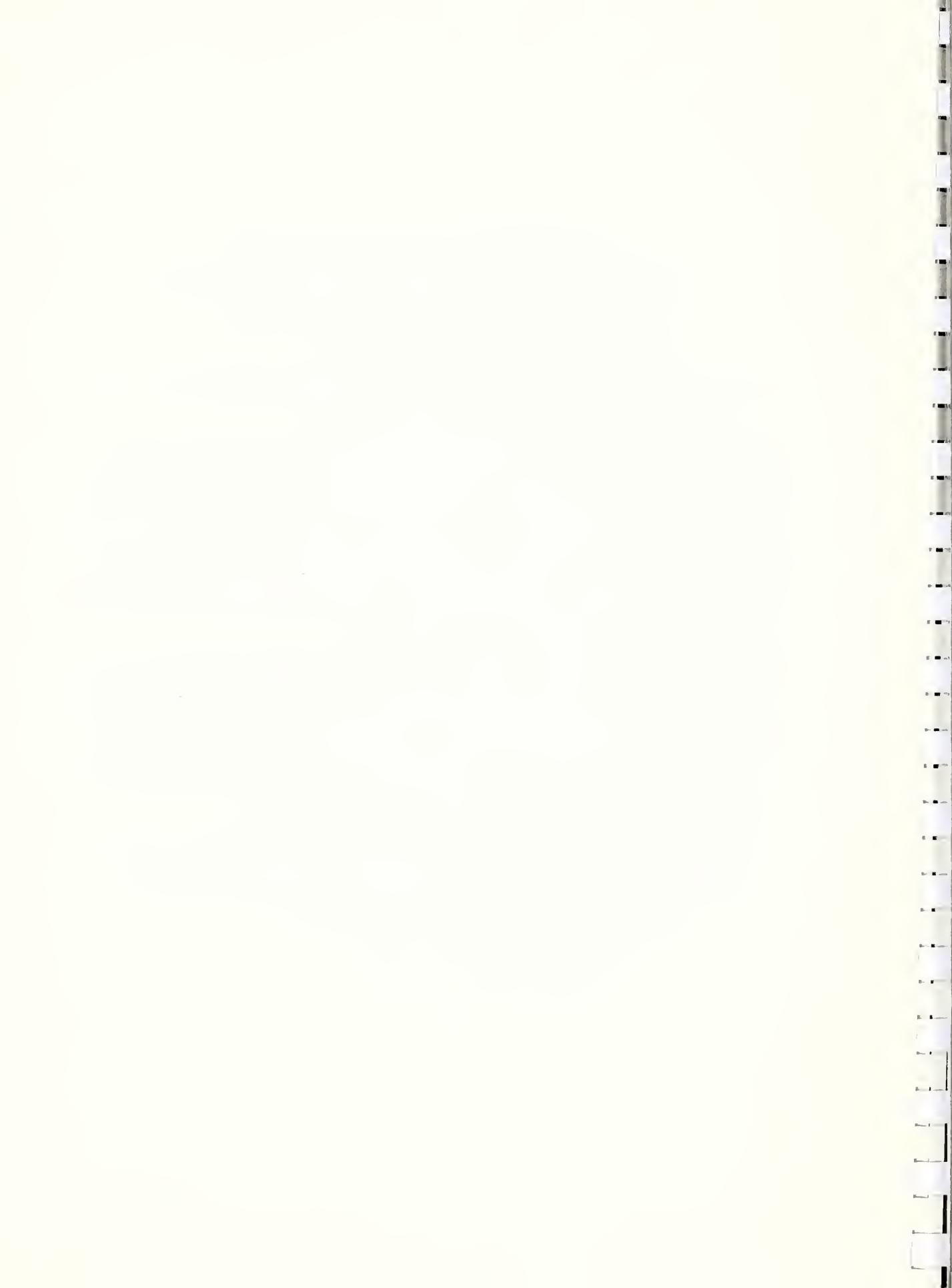
J. C. Webb, J. L. Sharp, and J. C. Benner

Objectives: To determine if the "signalling sound" produced by the male Caribbean fruit fly is required for pair formation.

Methods: Laboratory bioassays will be conducted to determine if the "signalling sound" is used as a sex attractant to attract female flies either alone or in combination with other attractants such as food, color, pheromone, etc. The bioassays will be conducted in a plexiglass chamber 2' x 2' x 7'. A 12-inch diameter cylindrical electric grid with 1/4 inch electrode spacing will be used as the trapping device and will be located inside one end of the plexiglass chamber. The attractant(s) will be placed in the center of the electric grid. The flies flying into the grid will be electrocuted and counted.

Results: In preliminary tests, food and water was placed inside the electric grid. Then, 100 males and 100 females were released in the opposite end of the chamber. Ninety-percent of the flies that were released flew into the grid and were killed. This demonstrated that the flies would go into the grid for an attractant such as food and water. Next, 50 male flies were placed inside the grid. Food and water were positioned both inside and outside of the grid; of the 100 females released in the bioassay chamber, an average of 47% were attracted into the grid.

Plans: To determine if the signalling sound is an important behavioral cue and useful in manipulating the fly in the laboratory or field.



Sound Production by Conotrachelus nenuphar: I. Morphological Description of Sound Producing Structures of Males and Females

T. Carlyle, C. Calkins, J. C. Webb and H. R. Agee

(Continuation of report 36 73(7-12))

Objectives: The location and description of all potential stridulatory areas of plum curculios with special emphasis on sexual dimorphism.

Methods: Detailed descriptions, measurements, drawings, and photographs of the sound producing structures and comparisons of morphological differences between males and females are being made with the aid of a scanning electron microscope. Sound production ability of these structures was verified by microsurgery and sound recordings.

Results: The primary sound producing structures consist of a file and a scraper. The file for both the males and females is located on the ventral surface of the posterior medial portion of the left elytron. The file of the male is approximately 1.36 mm long and 0.21 mm wide and consists of continuous ridges across its surface. The file of the female is approximately 1.2 mm long and 0.20 mm wide. This file consists of rows of teeth that run across its surface.

The scraper of both the male and female has been identified as the left tubercle on the 6th abdominal tergite. Measurements and surgical techniques have revealed that the right tubercle and secondary tubercles do not normally come in contact with the file during stridulation. The middle 1/3 of the file is normally struck by the scraper during stridulation. However, if that portion of the file is of the file is damaged, the insect will adjust the stroke to strike those portions both anteriorly and posteriorly to the damaged area. If the left tubercle is removed, the insect does not appear to be able to adjust to bring any of the other tubercles into contact with the file.

Plans: Unless sounds are discovered that are not produced by the identified structures, this phase of the study is essentially complete. Scanning electron photomicrographs and measurements of all the structures have been completed for the male. A few tests are planned to further verify the structures of the female.



Sound Production by Conotrachelus nenuphar:

II. Characterization and Reproduction of  
Sounds Produced in Response to Various Stimuli

J. C. Webb, C. Calkins, H. Agee, and T. Carlyle

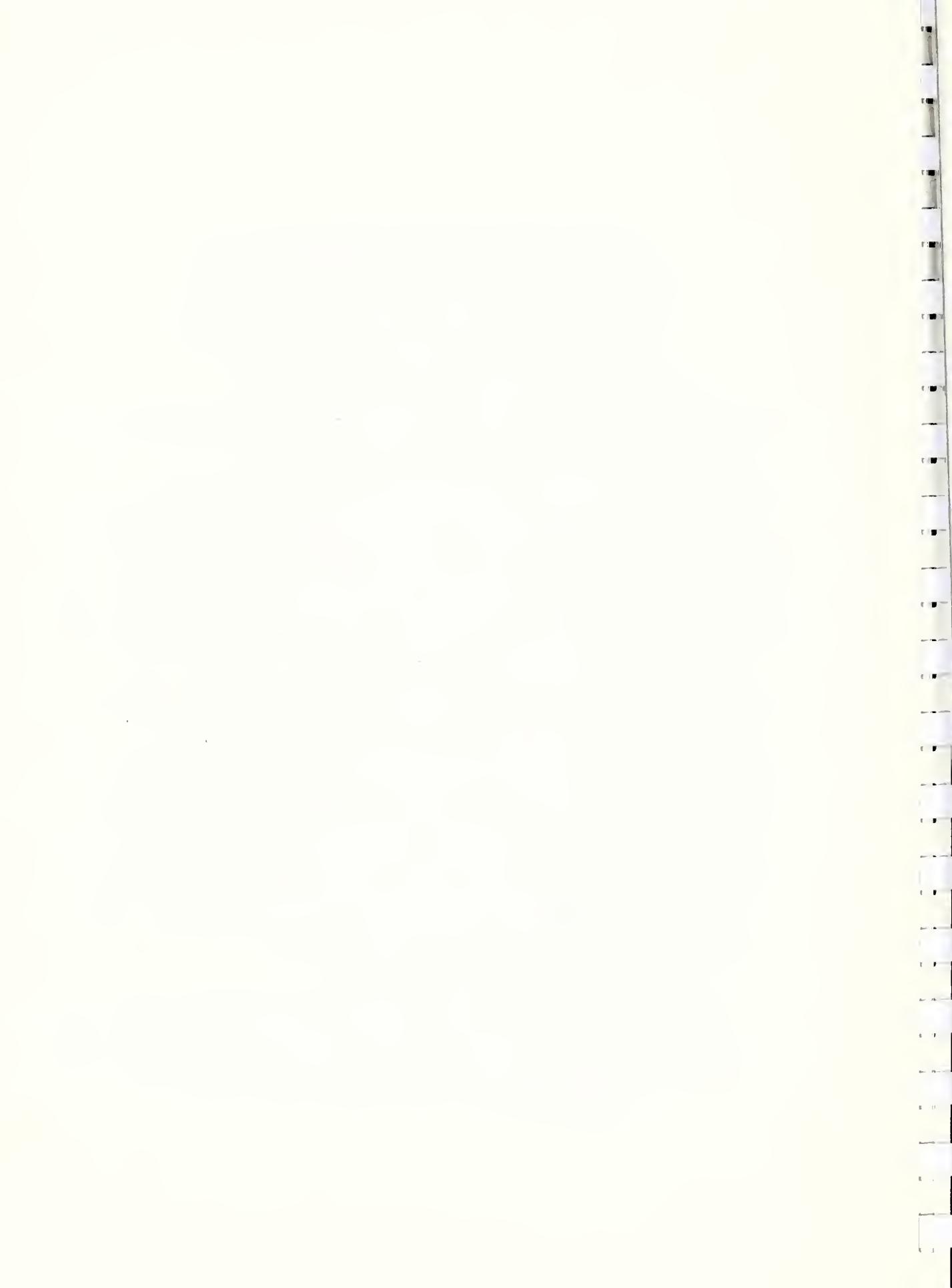
(Continuation of report 37 73(7-12))

Objectives: To analyze and identify the different sounds produced by adult plum curculios, and to ascertain the conditions necessary for the production of different types of sound.

Methods: The proper environmental conditions (temperature, light intensity, humidity, etc.) and stimuli necessary to elicit sound production will be determined. The sounds produced by various activities of the insect, such as territorial, aggression, and those sounds important to mating will be recorded and analyzed. The data obtained from these analyses will be used to determine if sound is used as an attractant by the plum curculio. The major equipment items used in this study are an anechoic chamber, condenser microphones, sound level meters, sound recorders and sound analysers.

Results: Certain conditions were observed to stimulate adult beetles to stridulate: exposure to bright sunlight while in petri dishes; near lethal high temperatures; carbon dioxide concentrations at subanesthetical levels; congregating numbers of beetles; and grasping of tarsi, legs or other body parts. Preliminary tests have shown that two strokes are made during stridulation. The average pulse duration of the first stroke was 0.6 second, and the second was 0.9 second. The average number of teeth strikes were 55 and 71 for the first and second strokes, respectively. The fundamental frequency of the primary tooth stroke rate appears to vary from approximately 400 to 2000 Hz. There is a secondary frequency of approximately 13 kHz that occurs when the scraper makes contact with a secondary ridge on the file. This secondary ridge occurs in approximately the center 1/3 of the file and is located about 1.25  $\mu$ m from the primary ridge.

Plans: Continued efforts will be made to identify different sounds and the conditions necessary to produce each type. Insects will be isolated according to sex to stimulate calling behavior. Sounds will be reproduced and played back to observe behavioral responses. If sound production is involved with mating behavior, recordings will be tested to see if the sounds can be utilized in trapping or mating disruption.



Sound Production by Conotrachelus nenuphar:  
III. Associated Behavior

C. Calkins, J. C. Webb, H. R. Agee, and T. Carlyle

Objectives: To determine the behavior of plum curculios, particularly mating behavior, as it is associated with sound production.

Methods: Detailed observations were made of adult behavior in the laboratory with the aid of microphones, speakers, recorders and a video camera.

Results: Three specific sounds were identified with specific behavior. A "stress" sound was produced whenever the individual was squeezed or when it came into contact with other beetles in restricted areas. This sound was produced by both sexes and may function to inhibit predation (47 73(1-6)). Another sound appears to have a territorial function, and it seems to be used primarily by the male. A third sound made exclusively by the male occurred after he had mounted the female but before copulation. The aedeagus was extended and stridulation occurred in a series of short strokes made continually. This lasted from a few seconds to several hours. The sound was terminated with the insertion of the genitalia. This behavior may function to stimulate the female and/or to inhibit the intervention of other males.

Plans: Observations and sound recordings will continue to be made until all aspects of these phenomena are understood and described.



Spectral Sensitivity of the Compound Eye  
of the Caribbean Fruit Fly

H. R. Agee and J. C. Webb

(Continuation of report 31 73(7-12))

Objectives: To determine the spectral sensitivity of the compound eye of the Caribbean fruit fly, Anastrepha suspensa (Loew) and relate these findings to behavior of the organism.

Methods: A calibrated light stimulus system capable of presenting monochromatic pulses of light energy (calibrated in microwatts/cm<sup>2</sup>) is used to stimulate the compound eye of the caribfly. Special micro-electrodes and electrophysiological techniques are used to detect and record the spectral sensitivity of the compound eye to light stimuli at wavelengths from 350 nm to 650 nm.

Results: The compound eye of the male and female Caribbean fruit fly displayed two peaks of sensitivity, a major peak at 500 nm (green) with a broad shoulder to 530 nm for the males and 540 for the females (green-yellow). A minor peak of sensitivity was found in the ultraviolet end of the spectrum at 365 nm.

Plans: The effect of age and rearing methods will be examined in later studies.



Effect of Increasing Levels of Gamma Irradiation  
on the Histological Structure of the Compound Eye  
on the Caribbean Fruit Fly

H. R. Agee, J. L. Sharp, J. C. Webb,  
B. J. Smittle<sup>1/</sup>, and M. L. Park

(Continuation of report 44 73(7-12))

Objective: To determine the effect of different levels of gamma irradiation on the structure of the compound eye of the Caribbean fruit fly.

Methods: Histological sections of stained preparations of the compound eye of the Caribbean fruit fly irradiated with 5 and 10 kR at different ages in air and nitrogen will be examined with the light microscope and these structures compared to those of nonirradiated flies of both sexes.

Results: Using the light microscope, no cellular damage to the compound eyes was detected when pupae were irradiated with 5 and 10 krads of gamma radiation 4 days before emergence.

Plans: Examination of other treatments will continue and electroretinograms of normal and treated flies will be compared.

1/ Research Entomologist, Insects Affecting Man Research Laboratory, Gainesville, Fla.



Analysis of the Influence of Color and Intensity of Light  
on Flight and Reproductive Activity  
of the Cabbage Looper Trichoplusia ni (Hübner)

W. K. Turner, N. C. Leppla, and V. Chew

(Extension of the study reported under 32 73(7-12))

Objectives: To conduct a detailed statistical analysis of specific activity criteria for cabbage loopers maintained under various combinations of light color and intensity.

Methods: Populations of 50 male and 50 female cabbage looper moths were held for 2 days under various colors and intensities of light. Thirteen treatments were applied consisting of continuous darkness (D) and 4 nocturnal intensities for each of three colors: green (G), ultraviolet (U), and red (R). Nocturnal levels of 0, 1, 10, or 100% (designated 1 through 4, respectively) of the diurnal intensity were used.  $\text{CO}_2$  produced by the moths during the final 24 hours of the tests served as an indication of flight activity. Relative reproductive rates were indicated by the percentage of females mated, spermatophores per mated female, volume of eggs, and percentage hatch.

The  $\text{CO}_2$  data were partitioned to yield four components of activity, consisting of (1)  $\text{CO}_2$  produced during the nocturnal, (2) ratio of nocturnal to diurnal  $\text{CO}_2$ , (3) increase in rate of  $\text{CO}_2$  production during lights-off transition (initial flight), and (4) increase in rate of  $\text{CO}_2$  production during the lights-on transition (startle). Statistical analyses included analyses of variance for overall treatment effects and effects due to color (c), intensity (I), and C x I. Differences for the four components were determined with Duncan's multiple range test. Analyses of variance and multiple range tests (within colors) were used for the reproductive criteria.

Results: Analyses of variance for the  $\text{CO}_2$  data revealed strong treatment effects (1% level) for all activity criteria. Color, intensity, and C x I effects were significant (1% level) for all criteria, with two exceptions: initial flight was weakly dependent (5% level) on intensity and not dependent on the C x I interaction. The more pertinent findings for the multiple range tests are as follows: For night activity, G<sub>2</sub> > all others, and U<sub>1</sub> < all except D; for the nocturnal to diurnal ratio, D, U<sub>1</sub>, and G<sub>4</sub> < all others; for initial flight, U<sub>1-4</sub> > all others; and for startle, U<sub>1</sub> > all others. The results of analyses of reproductive data are summarized in Table 1.

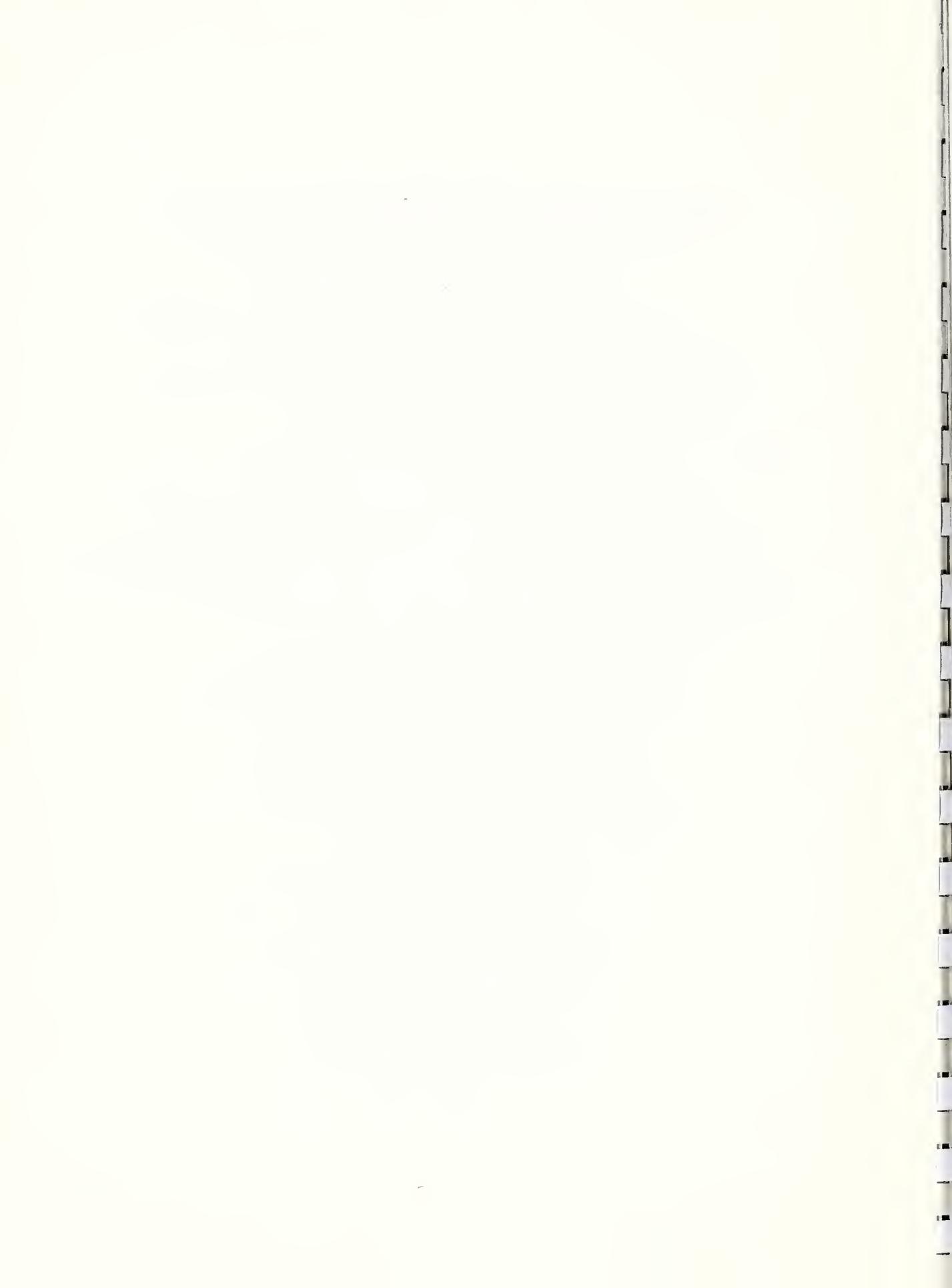
Plans: Work with the cabbage looper has been concluded; however, the approach will be applied to the Caribbean fruit fly in connection with the effects of colonization on successive generations. These techniques will be adapted to automated data acquisition and computer analysis.



Table 1. Reproductive activity of cabbage looper adults maintained under various combinations of color and nocturnal intensity of light.\*

Criteria	Color & Intensity											
	G				U				R			
	1	2	3	4	1	2	3	4	1	2	3	4
% females mated	a	a	b	c	a	b	a	b	-	-	-	-
Sper/mated female	ab	b	a	c	a	b	ac	bc	-	-	-	-
Eggs (volume)	a	b	ac	c	a	b	ac	bc	-	-	-	-
% hatch	-	-	-	-	-	-	-	-	a	c	ab	bc

\*Different letters in rows represent means that are significantly different (1% level) for intensities within a given color. Differences are not significant for rows without letters.



Morphology and Histology of the Compound Eye  
of the Beet Armyworm Moth, Spodoptera exigua (Hübner)

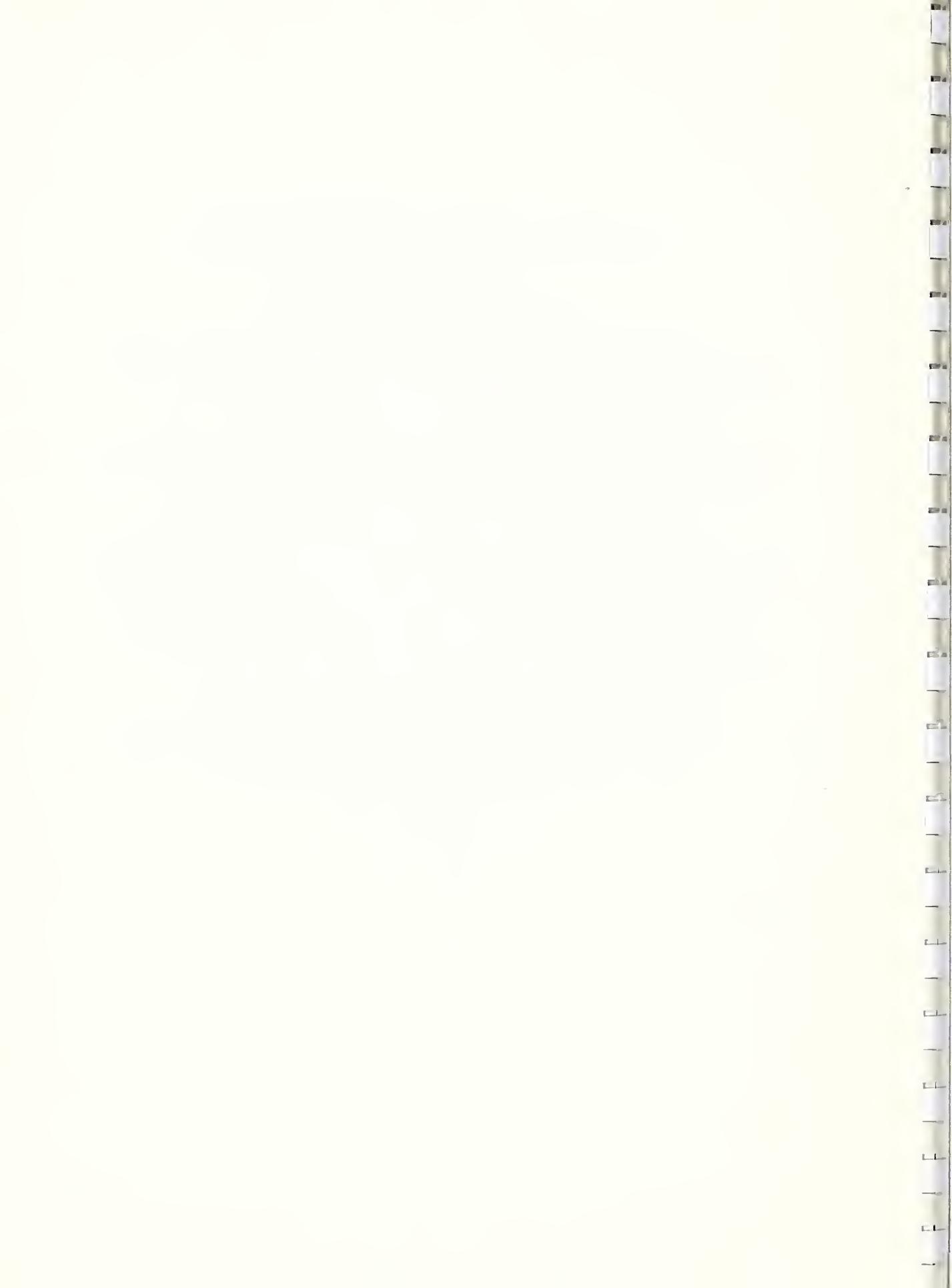
H. R. Agee

Objective: To determine the morphology and histology of the compound eye of the beet armyworm moth, Spodoptera exigua (Hübner) in order that electroretinogram studies can be conducted.

Method: The compound eyes of the beet armyworm moth were examined with the stereomicroscope and fixed and stained sections were studied using the light microscope to determine the cellular structure of the ommatidium unit.

Results: Each compound eye is composed of approximately 6-7,000 ommatidia. Each ommatidium is 18 microns wide at the lens and 260 to 290 microns long from lens to basement membrane. The eye has the cellular organization as described for the bollworm moth, Heliothis zea (Boddie). Pigment envelopes extend from the base of the cones to the distal end of the rhabdoms, being ca. 140 microns long. The 6-8 retinula cells per ommatidium form a closed rhabdom ca. 88 microns long.

Plans: The histological information developed for the compound eye of this insect was used to make electroretinogram studies on the sensitivity of the compound eye to light from 9 different colors of fluorescent lamps.



Sensory Response of Compound Eye of the Beet Armyworm  
Moth, Spodoptera exigua (Hübner)  
to Light From 9 Colors of Fluorescent Lamps

H. R. Agee, E. R. Mitchell and J. M. Stanley

Objective: To determine the relative effectiveness of light from 9 different colors of 15 watt fluorescent lamps for stimulating the beet armyworm moth compound eye receptors. This information was needed as a guide for selecting the lamp colors most likely to be effective for surveying populations of this insect in the field.

Methods: Electroretinogram responses from the compound eye of 2-3 day old lab reared moths were recorded using techniques developed specifically for these insects. The light stimulus consisted of a pulse of light at a duration of 0.11 sec at a pulse rate of 1 per 4 sec at the full intensity of a 1 cm<sup>2</sup> surface area of a 15 watt fluorescent lamp operated at 320 ma and 120 VAC. The lamp was positioned 1 meter from the insect eye. The nine lamp colors were photogreen, strontium blue, blue, boll weevil green, BL, BLB, gold, pink, and red.

Results: Electrophysiological data indicate that photogreen, strontium-blue, blue, and boll weevil green were the most effective lamps for stimulating electroretinogram responses while red was least effective. The lamps stimulating the greater physiological response should be more effective for attracting moths from greater distances in the field.

Plans: Light traps were operated using green (photogreen lamp factory substitute), blue, strontium blue, and BL (standard insect survey lamp) to test the effectiveness of the lamps in the field for survey potential.



## Attraction of Beet Armyworm Moths to Electromagnetic Energy

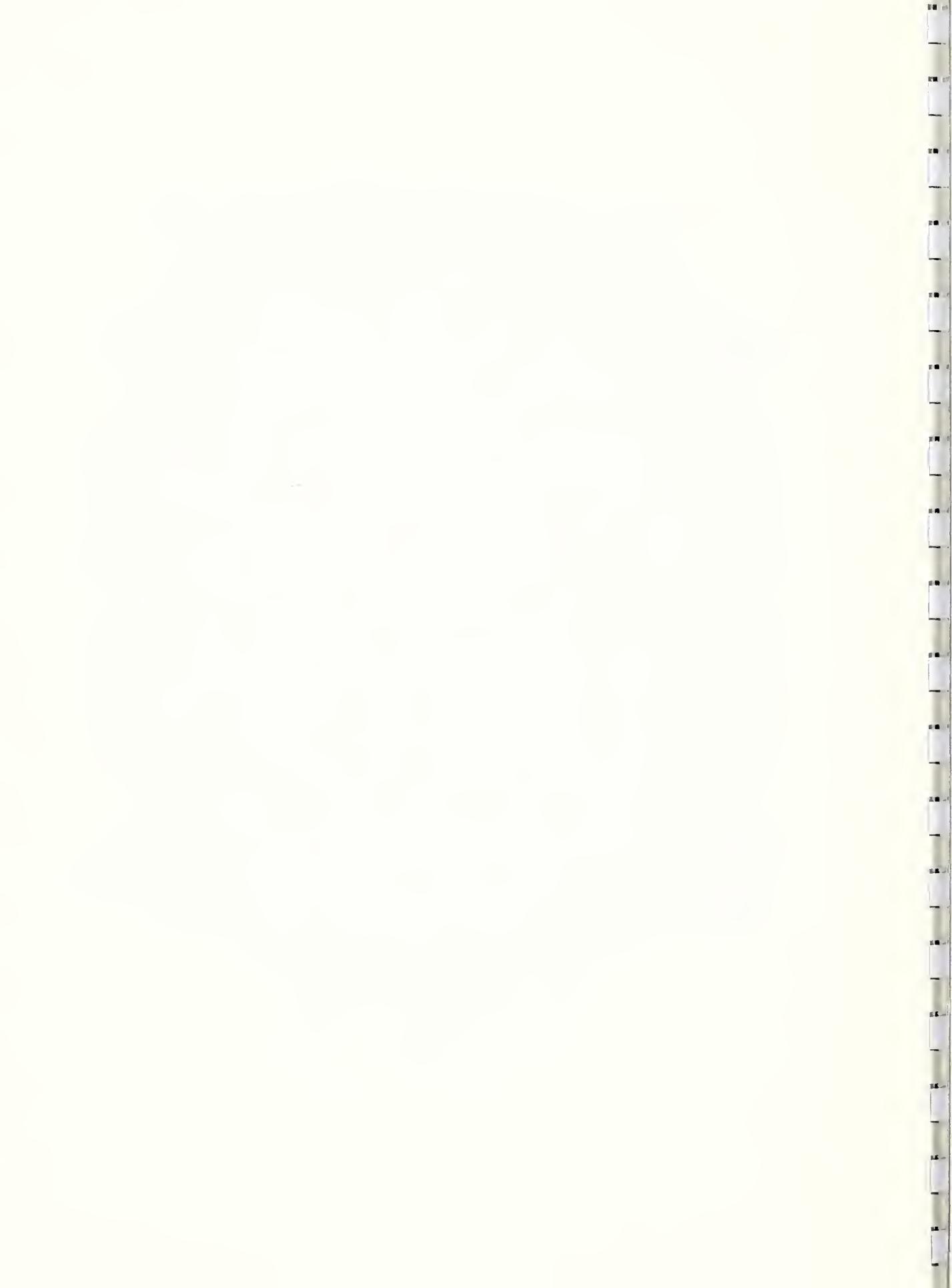
J. M. Stanley, E. R. Mitchell, and H. R. Agee

Objective: To determine the relative attraction of beet armyworm moths, *Spodoptera exigua* (Hübner), to the radiation from lamps that have elicited a significant electrophysiological response from these moths in laboratory tests.

Methods: Survey light traps equipped with black light (BL), blue, strontium blue, and green 15-watt fluorescent lamps were operated in field locations near Hastings, Fla., where a large beet armyworm population existed. Traps were installed and rotated according to an approved statistical design. During one series of tests traps were operated with the lamps being the only attractant. During another series, 3 virgin female moths were added to each trap.

Results: When comparing the catches of beet armyworm moths in traps equipped with the 4 lamps alone, the BL lamps were found to be significantly better than the other three lamps in attracting moths. No significant difference was obtained in the attraction by the other three lamps. With the addition of virgin females to the lamps the BL-lamp-virgin female combination was found to be significantly better than the combinations of the strontium blue and green lamps with female moths.

Plans: From this work the BL lamp will be the lamp of choice for use in trapping the beet armyworm. Further study is desirable on correlation of electrophysiological data and field trapping results toward understanding insect attraction to electromagnetic energy.



Insect Antennal Esterases: Continuing Studies

S. M. Ferkovich, M. S. Mayer, and R. R. Rutter

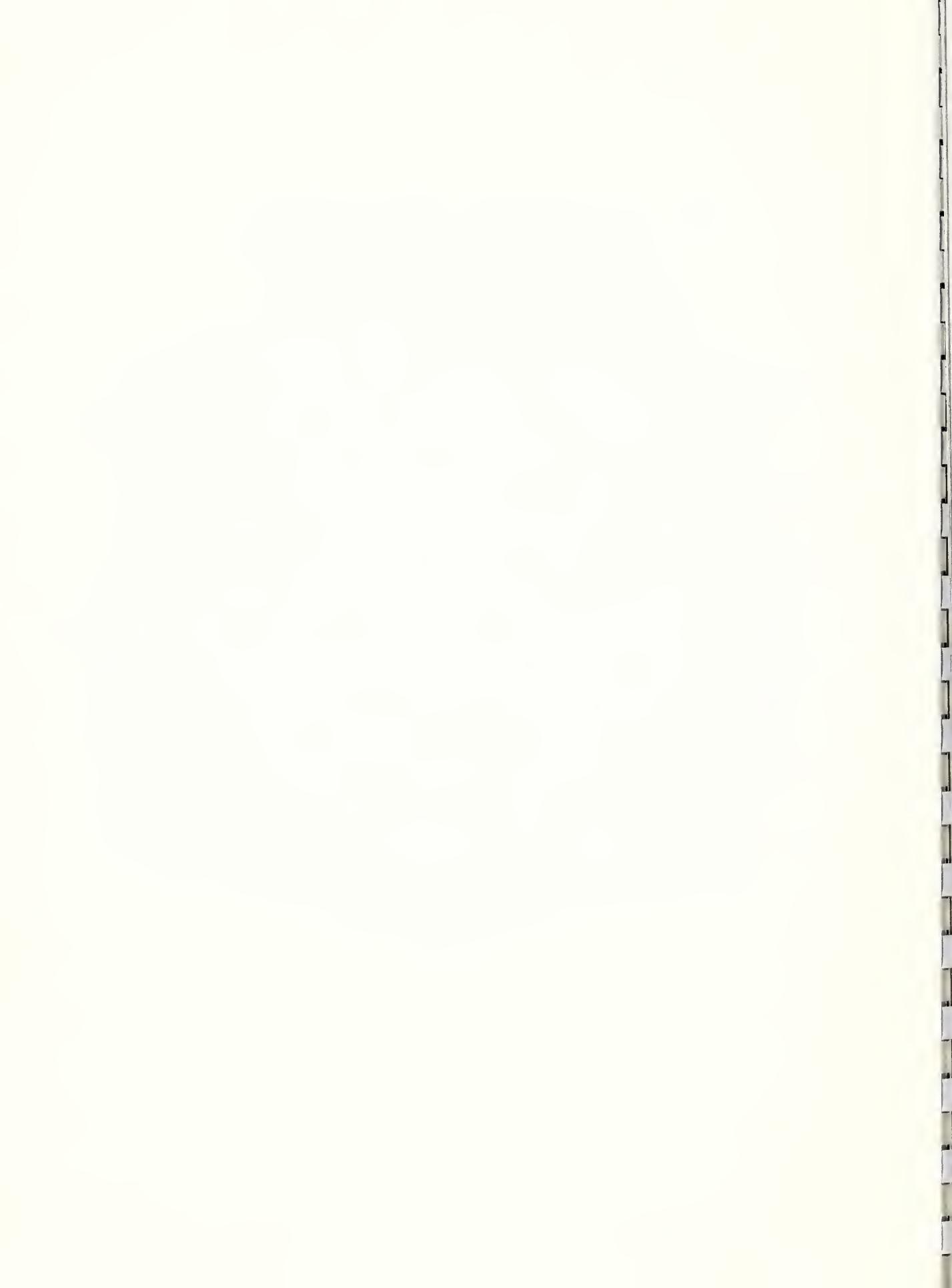
(Continuation of report 40 73(7-12))

Objectives: To ascertain if esterase activity in soluble antennal proteins is an integral part of the transduktory process, more data are needed to determine: (1) further evidence that enzymatic activity is located in membranes; and (2) to ascertain whether enzymatic activity can be inhibited by sulphhydryl group reactions.

Methods: Similar to report (49 73(1-6)) and use of transmission electron microscopy (TEM) of gel permeation fractions.

Results: The 1st group of enzymatically active fractions (those with a high enough molecular weight to be excluded from the gel) were collected, centrifuged and fixed for TEM examination. Vesicles resembling reformed membrane fragments were observed. The enzyme was reacted with n-Ethylmaleimide and Rose Bengal at the same time control tests were performed. The enzyme was destroyed by treatment with the sulphhydryl reagents, indicating that sulphhydryl groups are necessary for enzyme activity. A control reaction without inhibitor, exhibited an increased rate after sitting in a dilute buffer solution for a few hours. This increase could be due to rupturing of membrane-bound enzyme (although other explanations are available). Most importantly a first order plot of product concentration vs. time was very similar to in vivo studies.

Plans: To continue investigations into the activity of this esterase and its implications in olfaction.



Studies on the Infrared Emission of the  
Cabbage Looper Pheromone

P. S. Callahan

Objective: In order to understand how the emissions from insect sex attractants and host plant odors couple to the insect antennae, the infrared emission of various insect pheromones and attractive plants must be known. This project will locate and plot the emissions.

Method: A Fourier analysis spectrophotometer was modified so that a monomolecular layer coated on an aluminum vibrating reed "modulator" can be vibrated in the infrared source of the instrument. The reed vibrator is set at the maximum (55 cps) and minimum (42 cps) cabbage looper antenna vibration frequency. The monomolecular coated vibrator is enclosed in a 4 cm path length evacuated chamber and the spectrophotometer operated in the emission mode to obtain the emission spectrum of the pheromone.

Results: The cabbage looper pheromone emits "bright" (maser-like) narrow band frequencies in the following range:

$$\begin{aligned} 55 \text{ cps} &= 905 \text{ cm}^{-1}, 1090 \text{ cm}^{-1}, \text{ and } 1275 \text{ cm}^{-1} \\ 42 \text{ cps} &= 980 \text{ cm}^{-1}, 1120 \text{ cm}^{-1}, \text{ and } 1260 \text{ cm}^{-1} \end{aligned}$$

The difference between the three ranges at the maximum and minimum modulation frequency is  $75 \text{ cm}^{-1}$ ,  $30 \text{ cm}^{-1}$ , and  $15 \text{ cm}^{-1}$ , respectively (Fig. 2). Rubbing the monolayer briskly with a clean silk cloth increases the emission output. The aluminum reed vibrator in a clean state, and with a monolayer of fall armyworm pheromone vibrated at 42 cps and 55 cps showed no emissions in the 7 to 14  $\mu\text{m}$  window. Both the cabbage looper pheromone and the fall armyworm pheromone, however, showed identical frequencies in the 2.7 to 5  $\mu\text{m}$  window when the monolayers were vibrated at 42 and 55 cps.

A formaldehyde monolayer vibrated at 128 cps which is the lovebug, Plecia nearctica, antenna frequency showed characteristic frequencies in the 2.7 to 5  $\mu\text{m}$  window. Fig. 1 shows one such frequency at  $2455 \text{ cm}^{-1}$  (4.07  $\mu\text{m}$ ). The lovebug is attracted to formaldehyde. A monolayer vibrated at a stable antenna vibration frequency shows not only the high intensity emission line, but also two side bands usually of opposite character--one down (absorption, A) the other up (emission, B). The side bands are always exactly  $20 \text{ cm}^{-1}$  wavenumbers on either side of the high intensity emission line. They are usually ca. 1/10 the strength of the center emission line.

Plans: Further work is in progress in which the molecule is irradiated with various wavelengths of IR, in order to find and plot frequencies in other regions of the spectrum.



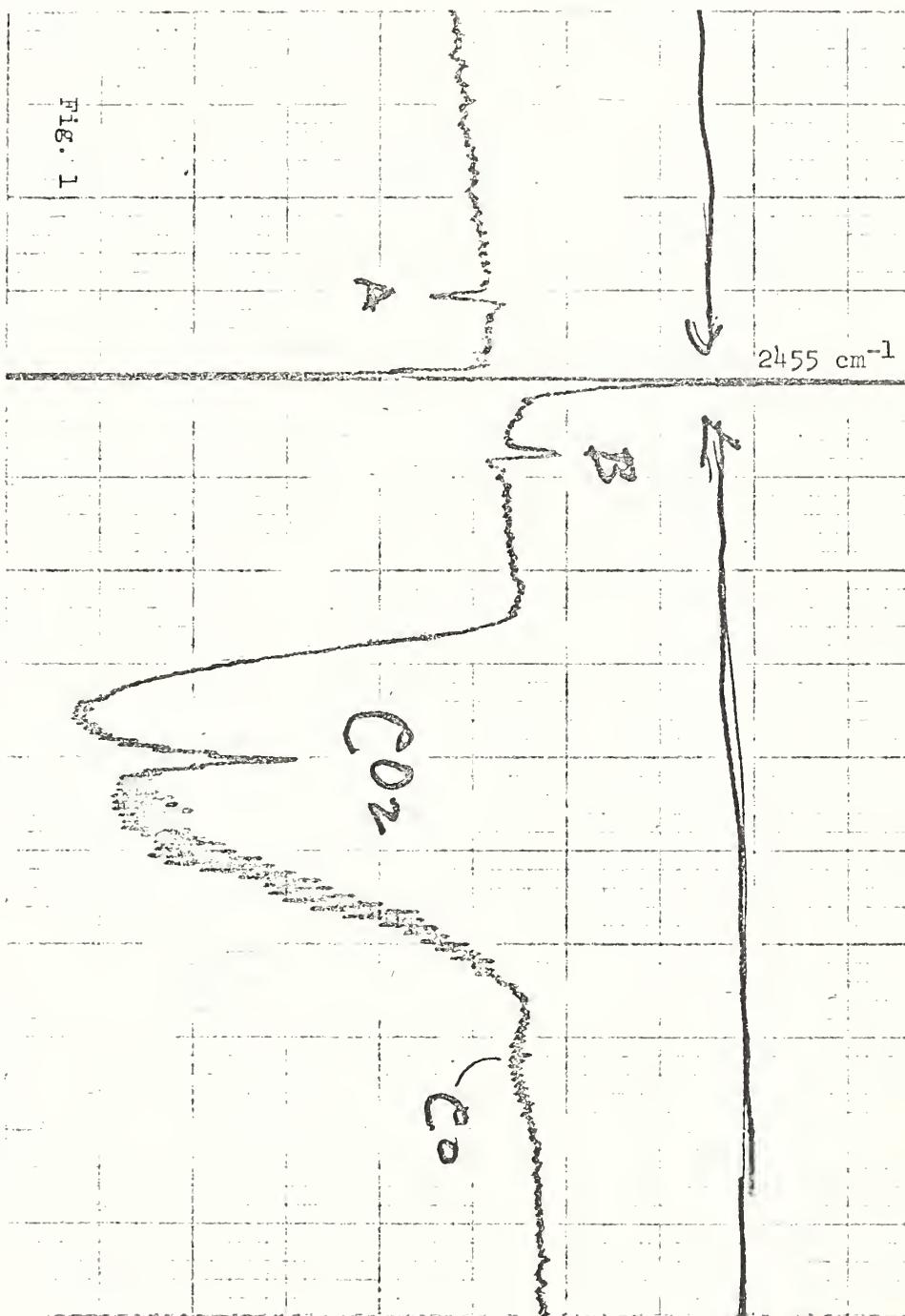


Fig. 1

Fig. 1 -- The 2455  $\text{cm}^{-1}$  (4.07  $\mu\text{m}$ ) line of a formaldehyde monolayer vibrated at the lovebug antenna frequency of 128 cps. Note the two equally spaced side band frequencies at A and B. They are 20  $\text{cm}^{-1}$  on either side of the emission band. Up is emission--down, absorption. The lovebug is highly attracted to UV irradiated formaldehyde.

Fig. 2 -- Cabbage looper pheromone "modulated" at 55 and 42 cps. A) Cabbage looper pheromone at 55 cps shows lines at 905  $\text{cm}^{-1}$ , 1090  $\text{cm}^{-1}$ , and 1275  $\text{cm}^{-1}$ ; at 42 cps 980  $\text{cm}^{-1}$ , 1120  $\text{cm}^{-1}$ , and 1260  $\text{cm}^{-1}$ . Note the spacing between the emissions at the two antenna modulation frequencies. These lines occur in the 7 to 14  $\mu\text{m}$  window as predicted by Callahan (1965). B) Fall armyworm pheromone modulated at 42 cps shows no emission. C) Clean vibrator check.

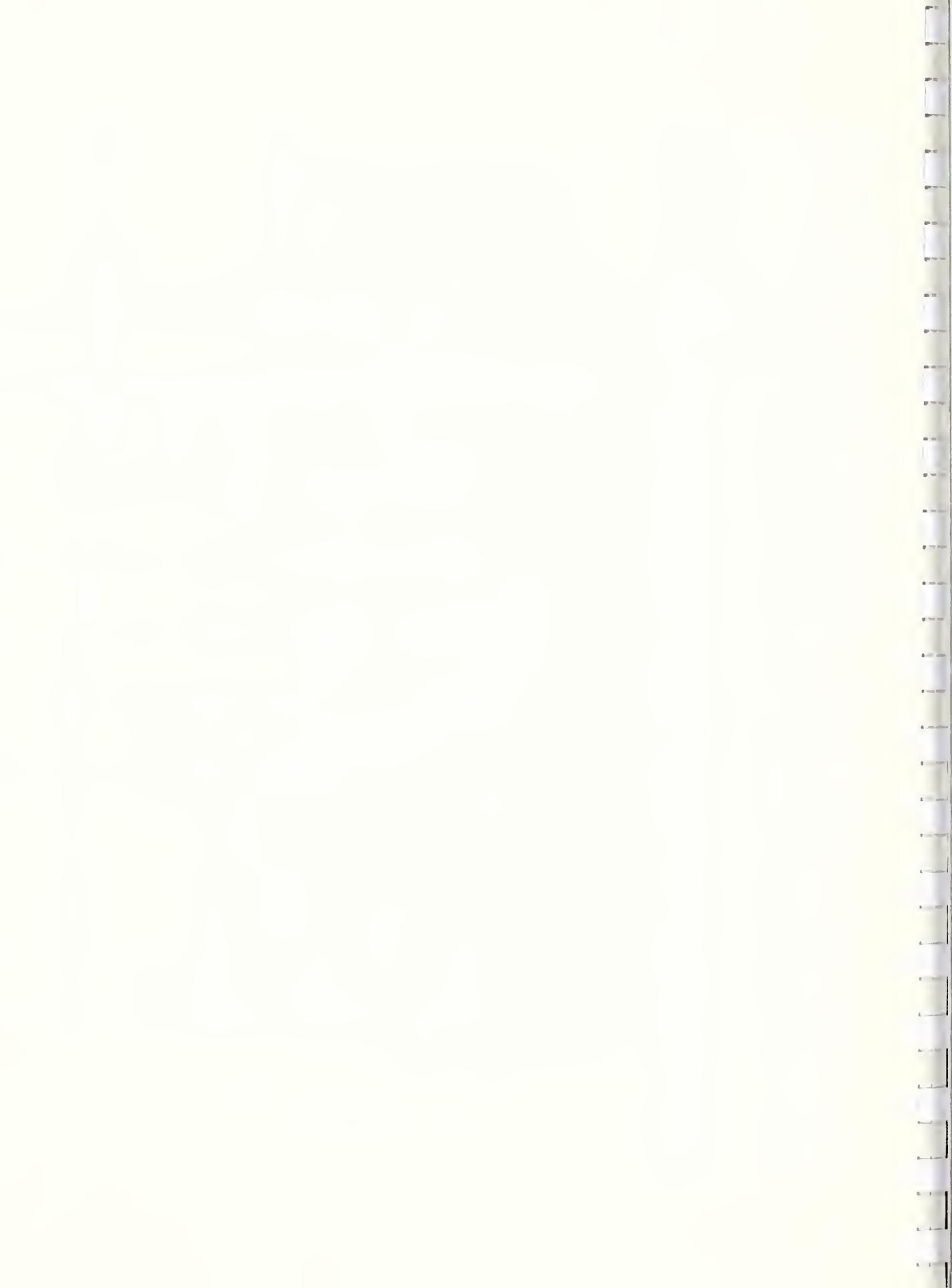
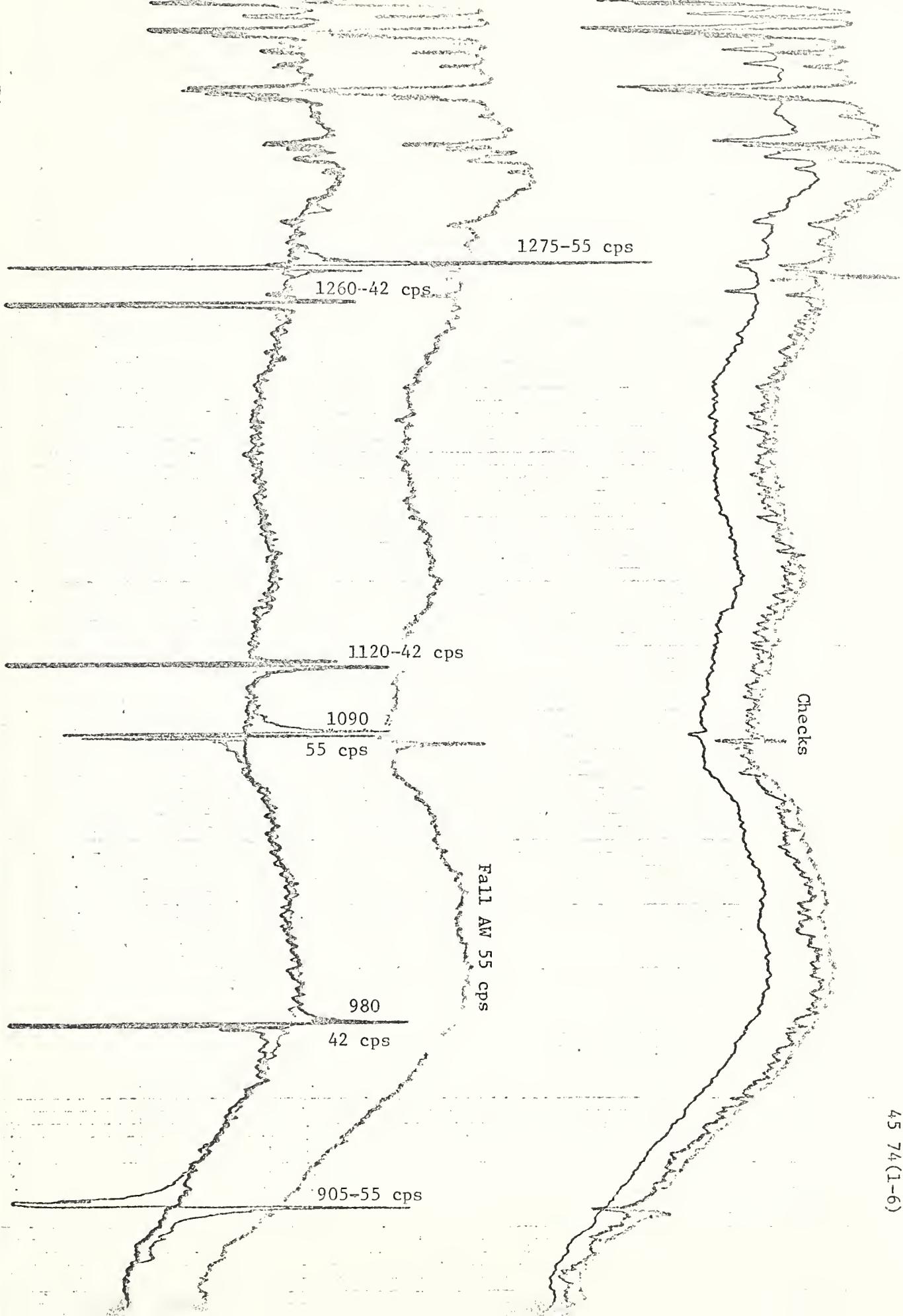


FIG. 2





Periodicity of Activity During Maturation  
In Populations of the Velvetbean  
Caterpillar Moth

N. C. Leppla and T. C. Carlyle

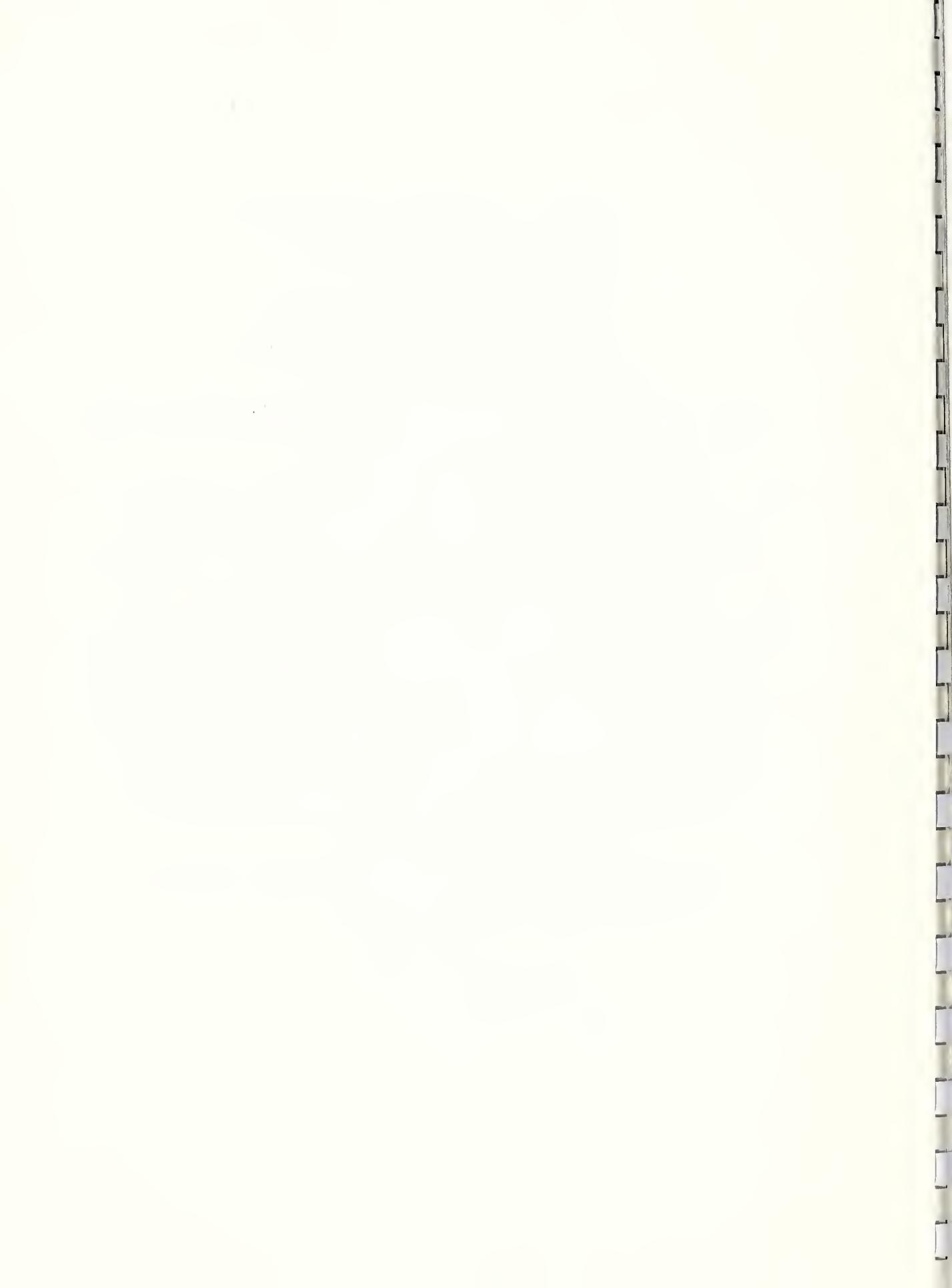
(Continuation of report 57 73(1-6))

Objective: To determine the diel rhythms of overt behavior including ambulation, calling, mating, oviposition, and feeding; and relate these activities to the age of monitored populations.

Methods: Behavior patterns associated with maturation and reproduction of moths were studied with an actograph and by direct observation. Histological analysis and dissection techniques were used to determine the influence of age and certain environmental factors on reproduction.

Results: Ambulation is arrhythmic for at least the 1st 3 days post-emergence, and on day 4 there is a characteristic reduction. Moths are entrained to nocturnality, with increased activity during the late scotophase, by days 7-18. Mating and ambulation occur with essentially the same circadian periodicity, except the reproductive age of a population ranges from 2 to 12-16 days. Courtship is initiated by female calling, but involves positioning and mutual assistance by both sexes. Copulation requires 30-180 min, ( $\bar{X} = 95$  min n = 42 pairs). Males may transfer 6 spermatophores and females may receive 5 or more, however 1-2 matings is typical for either sex. Oviposition and feeding are nocturnally arrhythmic, and isolated populations of either sex are completely aperiodic.

Plans: Histological sections and other data are being analyzed concomitantly with preparation of the manuscript.



## Activity Patterns of Individual Indian Meal Moths

L. L. Sower and W. K. Turner

Objectives: To observe the spontaneous activities of individual insects in the absence of immediate external stimuli.

Methods: Activities were scored automatically according to  $\text{CO}_2$  production using methods of Turner and Charity (1971) refined to allow observation of single insects. The automatic observations were supplemented by extensive visual observation.

Results: The spontaneous activities of 6 adult males were determined from 0-4 days following their emergence from pupae. Three distinct patterns of activity were apparent among the several insects: 1) periodic activity of long duration, 2) periodic activity of short duration; and 3) largely random bursts of activity during the scotophase. Each male appeared to reproduce its particular behavior pattern on a circadian basis.

Plans: These observations will be expanded.



Activity of Anastrepha suspensa (Loew)  
Related to Irradiation in Air vs. Nitrogen

J. L. Sharp, N. C. Leppla, and B. J. Smittle

Objectives: To determine if irradiation of pupae in nitrogen results in fewer deleterious effects on the activity of the fly than does irradiation in air.

Methods: Pupae of A. suspensa from our stock colony were irradiated 4-5 days before adult eclosion with  $^{60}\text{Co}$  at a dose rate of ca. 1 kR/min in air and nitrogen at doses of 5 or 10 kR. Controls were adults from pupae exposed only to nitrogen for 0.5 h and adults from pupae exposed to air. About 1 day after adult emergence, 20 males and 20 females from each treatment were put into cages provided food and water. The adults were kept in the cages in a cabinet at controlled conditions of 60-80% RH and 13:11 L:D cycle. Mortality readings were taken daily. Actographic recordings of overt activity were taken for 6 days after the flies had been confined in their cages for 8 days because little activity occurred during the first week of their adult life.

Results: During the tests, most deaths occurred (20% mortality) with male + female (10 kR + air) followed by male + female (air) and male + female (5 kR + air), each 13% mortality; least mortality occurred with male + female (nitrogen), 8%. When activity was adjusted for mortality and averaged from 6 days of recordings, male + female (5 kR + nitrogen) were most active (1082 counts); next were male + female (nitrogen), 810 counts; male + female (air), 437 counts; male + female (10 kR + nitrogen), 329 counts; male + female (10 kR + air), 64 counts; and male + female (5 kR + air), 51 counts.

The preliminary results indicate that the use of nitrogen rather than air in irradiation at high doses of young puparia will yield adults that are less adversely affected with regard to mortality and activity by the irradiation treatment.

Plans: Additional tests are planned to compare the effects of atmosphere during irradiation of older pupae and of adults.



Changes in Flight of Anastrepha suspensa  
Induced by Gamma Irradiation

J. L. Sharp and B. J. Smittle<sup>1/</sup>

Objective: To determine the effects of gamma irradiation on flight ability of A. suspensa.

Methods: The pupae were irradiated 2 days before adult eclosion at doses up to 20 kR (ca. 4X the 100% sterility dose). Wingbeat frequency studies of irradiated vs. nonirradiated males and females showed few changes in adult performance regardless of the dose, age, or sex. Flight mill studies are being carried out with the adults irradiated with 10 kR as pupae 2 days before adult eclosion. Tests were performed when they were 1-2, 5-6, and 11-12 days old.

Results: Preliminary results are shown in Table 1. Data for 11-12 day old flies have not been analyzed.

Plans: At least 2 more replications of the flight mill tests are planned.

1/ Research Entomologist, Insects Affecting Man Research Laboratory, Gainesville, Fla.



Table 1. Flight ability (mean) of irradiated and nonirradiated flies flown for 3 hr<sup>1/</sup>.

	Flight time (min)	% time flying	Distance (m)	Flight velocity (m/min)	No. flights/hr
<u>1-2 days old</u>					
Normal ♂	56	30	1818	32	100
Normal ♀	82	45	2177	28	146
Irradiated ♂	52	28	1686	32	67
Irradiated ♀	84	46	2614	28	100
<u>5-6 days old</u>					
Normal ♂	67	36	2676	39	40
Normal ♀	105	37	4167	39	39
Irradiated ♂	92	49	3771	41	30
Irradiated ♀	100	54	3739	41	37

1/ Each mean based on 9 flies.



The Flight Ability of Trichoplusia ni (Hübner) in the Laboratory

J. L. Sharp, J. R. McLaughlin, T. R. Ashley, and D. R. Bennett

(Continuation of report 47 73(7-12))

Objectives: To determine the flight ability of T. ni in the laboratory.

Methods: Cabbage loopers from our stock laboratory colony were fastened to the flight mill system and allowed to fly. Nonmated 1-day-old males were tested vs. 1-day-old virgin females at 50% RH and 21.1, 27.7, and 32.2°C. Also, nonmated males and virgin females were flown each day at 50% RH and 26.7°C for ca. 60 min when they were 1, 2, 3, 4, and 5 days old. At the same RH and temperature, 4-5-day-old virgin females were tested vs. mated females for 6 hours.

Results: Both sexes tended to fly best at 21.1°C and 50% RH, and males tested at 21.1°C and females flown at both 21.1 and 26.7°C were significantly better fliers than males and females tested at 32.2°C. Females had similar flight capability when tested at ages from 1 to 5 days at 26.7°C and 50% RH; males displayed significantly better flight ability at 2 and 3 days than when they were 5 days old. Both 1- and 5-day-old females outperformed males tested at those ages. Virgin females were significantly better fliers than mated females (4-days old) when tested for 6 hr at 26.7°C and 50% RH.

Plans: These aspects of this research have been completed, and a manuscript is being prepared. However, tests of wild strains vs. the laboratory colony are being readied. Also, tests will be carried out to determine the movement of marked and recaptured moths.



Flight ability of Pseudoplusia includens (Walker) in the Laboratory

J. L. Sharp, J. R. McLaughlin, D. R. Bennett, and T. Antonio

Objectives: To determine the flight ability of P. includens in the laboratory.

Methods: Soybean loopers from our stock laboratory colony were fastened to the flight mill system and allowed to fly. When nonmated males and virgin females were 1-day old, they were flown for ca. 1 h at 50% RH at 21.1°, 26.7° and 32.2°C. Also, nonmated males were tested vs. virgin females for 1 h at 50% RH and 26.7°C when they were 1, 2, 3, 4, and 5 days old.

Results: Preliminary results at different ages are shown in Table 1. The mean distances flown by 1-day-old males and females at 50% RH and 21.1°, 26.7°, and 32.2°C were 1459 and 1642, 1714 and 881, and 640 and 1193 m, respectively.

Plans: To investigate the flight ability of mated females vs. virgin females during 6 hours; to carry out more tests of nonmated males and virgin females at the 3 temperatures; to fly wild moths (collected as larvae in the field and reared in the laboratory) and conduct mark, release, recapture studies in the field.

Table 1. Flight data of P. includens at different ages.

Categories	Age (days) Male - Female				
	1	2	3	4	5
Distance (m)	803-1734	494-1134	329-1061	817-656	566-492
$\bar{X}$ flight velocity (m/sec)	0.8-0.9	0.8-0.8	0.7-0.9	0.9-0.9	0.9-0.9
% time flying	23-54	17-41	13-36	28-17	16-16



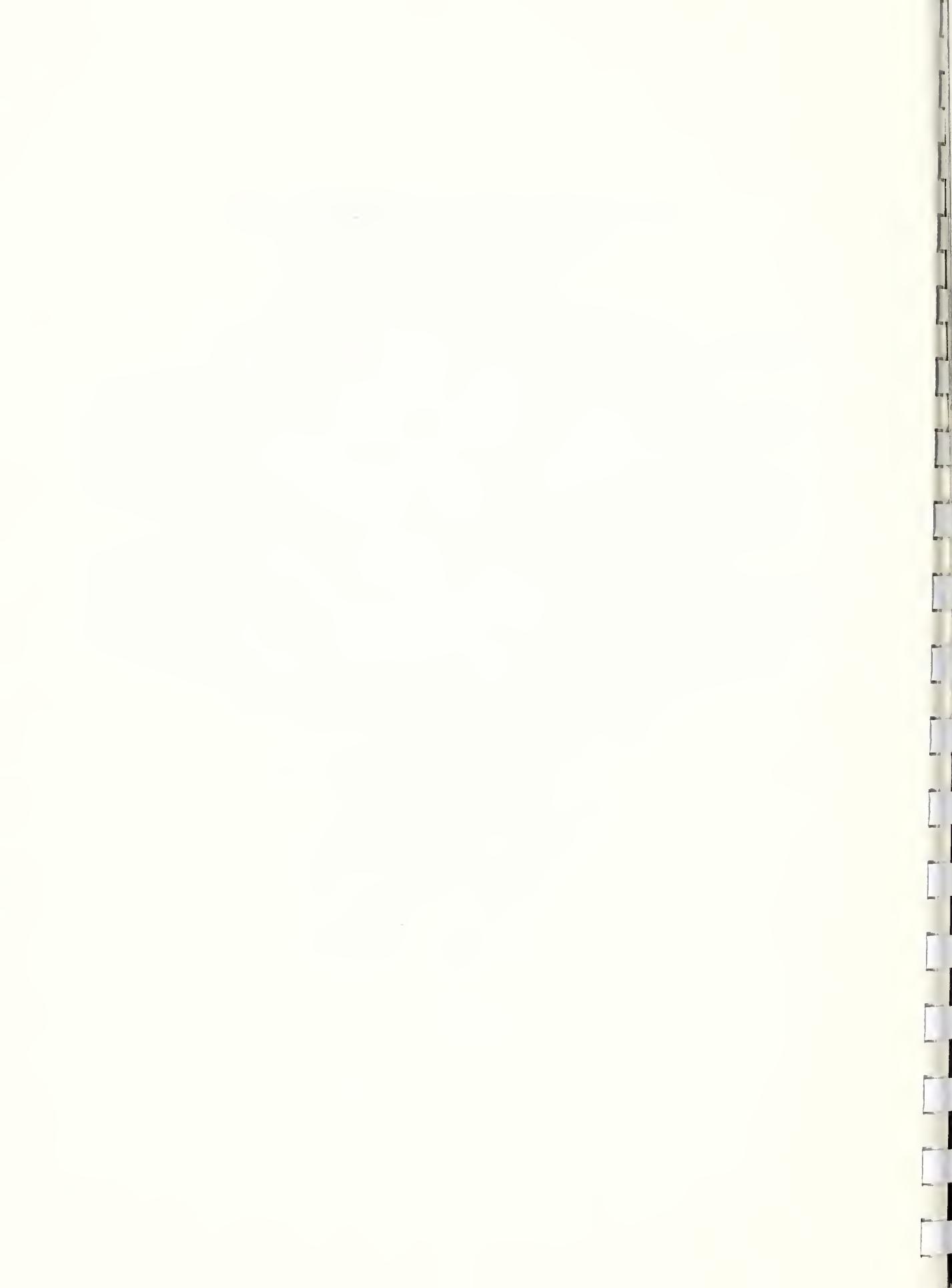
Quality Measurements of Anastrepha suspensa  
Reared on Two Different Diets

J. L. Sharp

Objective: To maintain a colony of A. suspensa reared in the laboratory on a corncob substrate diet formulation.

Methods: A population of A. suspensa reared on corncob-based larval diet has been established at this laboratory. This population originated from a colony established by A. K. Burditt, Jr., Miami. This strain of corncob-reared flies will be compared with flies reared on bagasse diet. (A subcolony of adults reared from bagasse-based diet had already been established at this laboratory. It originated from pupae provided by R. M. Baranowski, Homestead, Fla.)

Plans: Flight ability and propensity, competitiveness, and actographic measurements will be made for corncob- and bagasse-reared flies. Also, longevity and fecundity studies will be carried out. These tests, as well as those among other strain selections and treatments are designed to develop and demonstrate quality criteria for mass-produced flies.



Computer Program to Analyze Flight Mill Studies

T. R. Ashley, J. Sharp and D. L. Chambers

Objective: This program provides a detailed analysis of insect flight ability as recorded from a flight mill onto magnetic computer tape by an 18-channel event recorder.

Methods: The event recorder records at 0.5 sec intervals whether or not activity has occurred on any of 18 mills. The computer program examines the amount and distribution of activity for each mill.

Results: For each of the 18 mills the following values are calculated: time in min flying and resting, % of time flying and resting, total number of revolutions, distance traveled (m), mean flight velocity (m/min), and the maximum flight velocity. The flight length (min), flight number, time of occurrence, distance traveled (m), and the number of revolutions are printed out for the flight having the maximum flight velocity. The remaining output consists of frequency and percentage distributions for flight lengths, distances, and velocities. All of the above mentioned values are passed to a statistical subroutine which calculates their sum, mean, variance, standard deviation, standard error, 95% confidence interval, coefficient of variation, and range.

Plans: A manuscript is currently being prepared.



Role of Dispersal and Life History Characteristics in the  
Population Dynamics of the Almond Moth, Cadra cautella  
in a Citrus Pulp Warehouse

D. W. Hagstrum and Jane Sharp

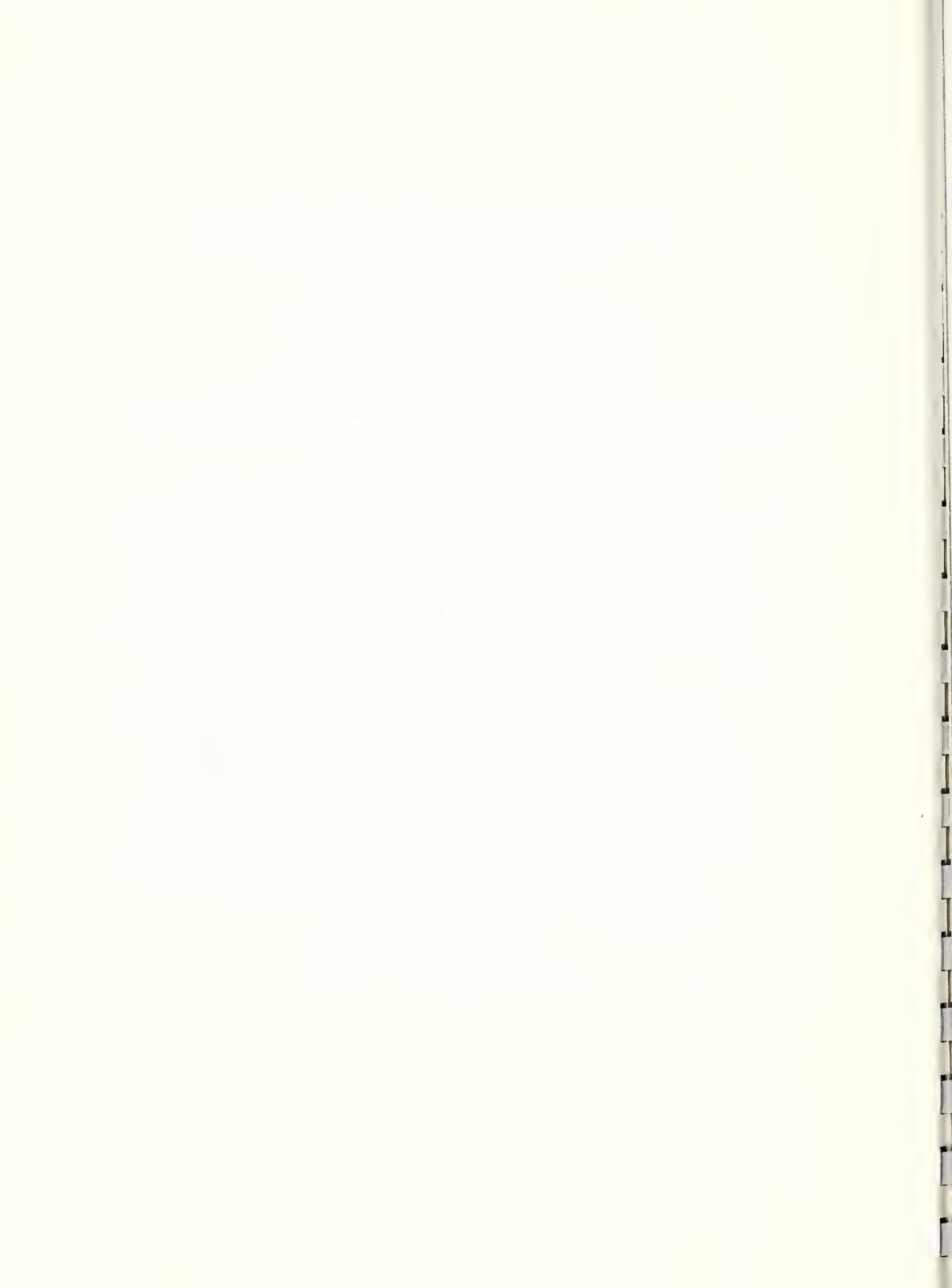
(Continuation of report 50 73(7-12))

Objective: To identify and describe aspects of the insect's behavior and life history that might be used to improve current methods of control or develop new ones.

Methods: The larval population of the almond moth in a citrus pulp warehouse was sampled weekly using artificial pupation sites. The incidence of diapause among samples was determined. Light traps were used to sample parasitic hymenoptera from the 23rd week of storage onward. Moths were reared at 8L:16D, 10L:14D, 12L:12D, 14L:10D, 16L:8D and in darkness to determine the role of photoperiod in the initiation of diapause. Small bags of citrus pulp were interspersed among larger bags and were recovered later to determine the age structure of the immature population.

Results: The population trends during the 1st two generations were similar to those observed during the previous year. However, between the 2nd and 3rd generations, the population decreased rather than increasing as in the previous year. This decrease was accompanied by a large increase in the number of parasitic hymenoptera, probably Bracon hebetor. The absence of parasites during the previous year could have resulted from malathion applications. As in the previous year, the incidence of diapause increased throughout the storage period and was inversely related to the total number of larvae captured. Results of studies on diapause are not yet available. Those food packets examined contained few larvae.

Plans: Studies on diapause and population dynamics will be continued.



Role of Constant and Fluctuating Temperatures in  
Determining the Behavior and Development Time of  
Stored-Product Insects

D. W. Hagstrum

(Continuation of report 52 73(7-12))

Objectives: Differences in an insect's response to constant and fluctuating temperatures are often important to the prediction and manipulation of behavior, life history or physiology. This study seeks to identify and quantitate these differences.

Methods: The development times of the red flour beetle, Tribolium castaneum were determined for 8 populations of 50 insects that were exposed to constant temperature. Eight populations of similar numbers were exposed to sinusoidal temperature cycles with 10°C ranges. Mean temperatures of 22.5, 25, 32.5 and 35°C were compared. The influence of temperature fluctuations on oviposition rate was also determined using the same 8 temperature conditions. For each temperature condition the oviposition rates of 20 females on 3 successive days were observed.

Results: For beetles exposed to constant and fluctuating temperatures with the same means, the developmental times and oviposition rates were significantly different. At 22.5 and 25°C developmental times and oviposition rates were highest at fluctuating temperatures and at 32.5 and 35°C they were highest at constant temperatures.

Plans: Studies will be expanded to include other temperatures. Further work will be initiated to determine the reason for differences in the response of these insects to the two environments.



Comparative Life History of "Wild Type"  
and Colonized Caribbean Fruit Flies

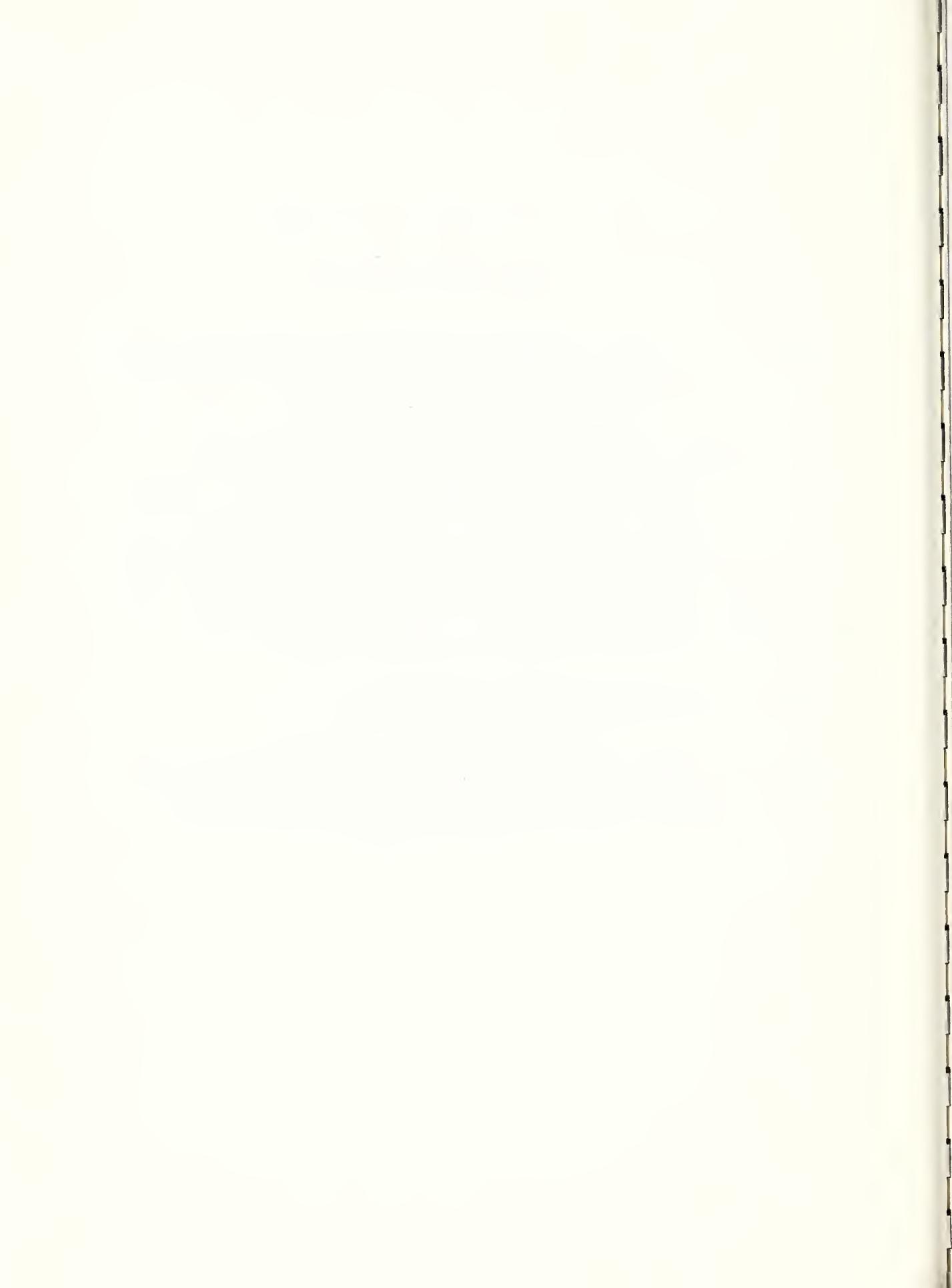
N. C. Leppla and M. D. Huettel

Objective: To compare a "wild type" population of Anastrepha suspensa (Loew) that is adapting to colonization with an established laboratory strain for the duration and survival of each stage of the life cycle.

Methods: Three equivalent populations were established using 1-2000 pupae collected from the original gene pool in Homestead, Fla. "Wild types" I and II, and an inbred laboratory colony have been isolated and maintained at  $80\pm2^{\circ}\text{F}$  and  $65\pm5\%$  RH, with a 12-hr light: 12-hr dark photo-period. The inbred reference culture has been lab-reared for several years and will provide a standard of comparison for measuring the processes of adaptation in the other 2 populations. Relative fecundity, egg viability, duration of the larval and pupal stages, pupal weights, and yields of pupae and adults (by sex) will be recorded for each generation. Variations in these parameters will identify the source and magnitude of selection on the "wild type" colony.

Results: The parent generation of each caribfly colony is viable under the established rearing protocol.

Plans: This is part of a cooperative project conceived as a series of independent studies based on a common research theme and supported by a set of available caribfly colonies. The extent and duration of the research effort will depend on individual assessments (probably 10-15 generations).



Genetic Variation Between Populations of the  
Plum Curculio, Conotrachelus nenuphar (Herbst)

M. Huettel, and C. Calkins

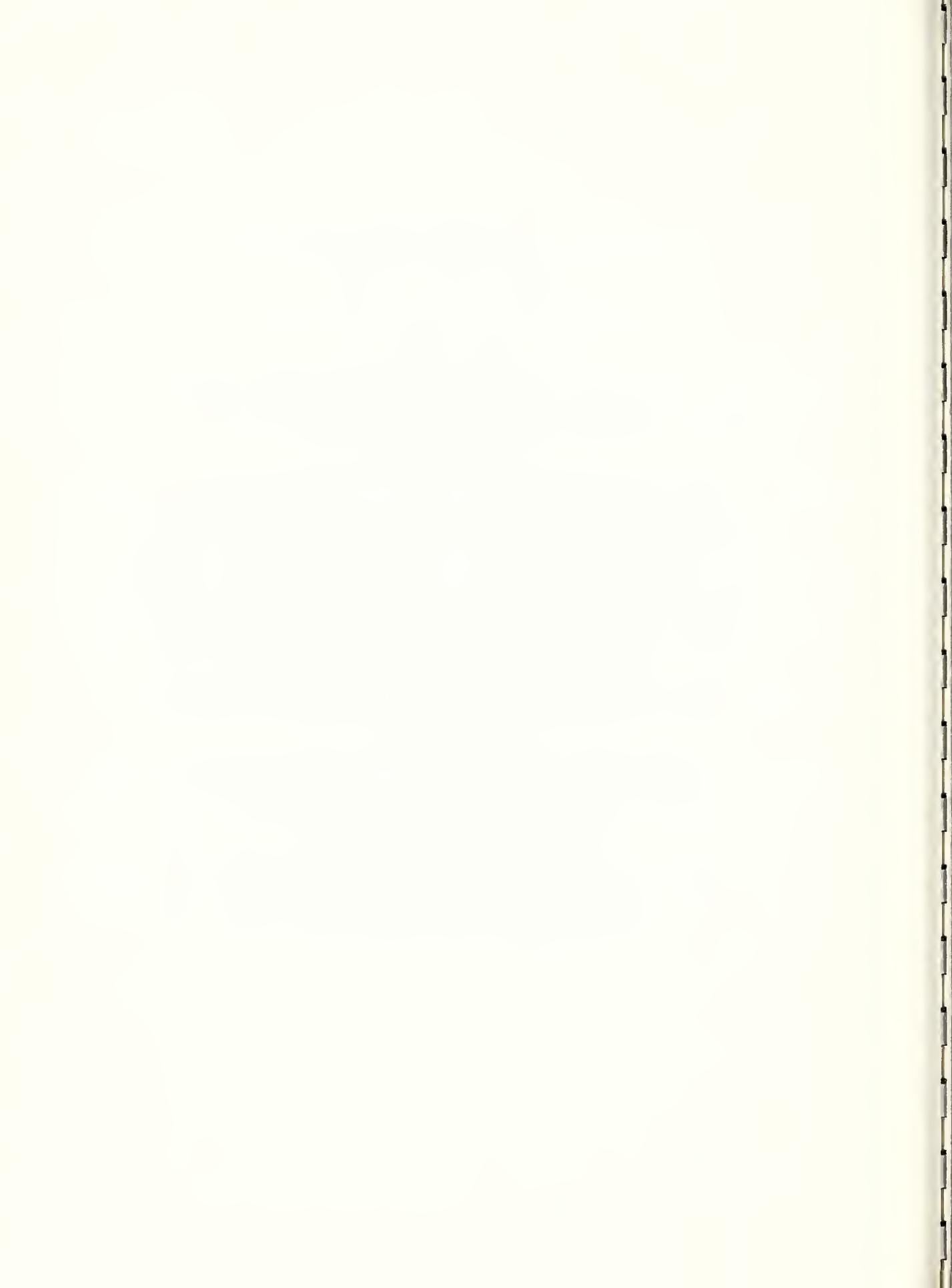
(Continuation of report 54 73(7-12))

Objectives: A study has been undertaken to investigate dispersal patterns and the possible existence of host and/or geographic races in the plum curculio.

Methods: Spatial variation in isoenzyme alleles at various loci will be analyzed at 4 levels. (1) All wild plum thickets on the Tall Timbers Research Station, Leon County, Fla., were mapped during the spring bloom period (46 thickets total). Fruit samples from as many of these thickets as possible were taken at several times during the season. Samples will be analyzed for close spatial and temporal variation. (2) Samples from wild plums were taken several miles apart along several transects through north Florida. (3) Samples were collected from 10 southeastern states from wild plums and, where possible, sympatric cultivated hosts (peaches and plums). (4) Samples are being provided by cooperators from the northern portion of the range where the univoltine race predominates. Genetic distances between relevant populations will be calculated.

Results: Electrophoresis of samples of plum curculio larvae is being carried out at the present time. No generalization may as yet be made about the nature of geographic or host-related variation in this species.

Plans: A detailed picture of the genetic structure of the plum curculio in terms of spatial, temporal and host-related variation should emerge from this study. Evidence of partial or complete reproductive isolation at any level of organization of the species may be found. Detailed behavioral studies and hybridization tests may then be necessary.



Use of Isoenzyme Markers to Evaluate the Performance  
of Lab-Reared Insects in the Field

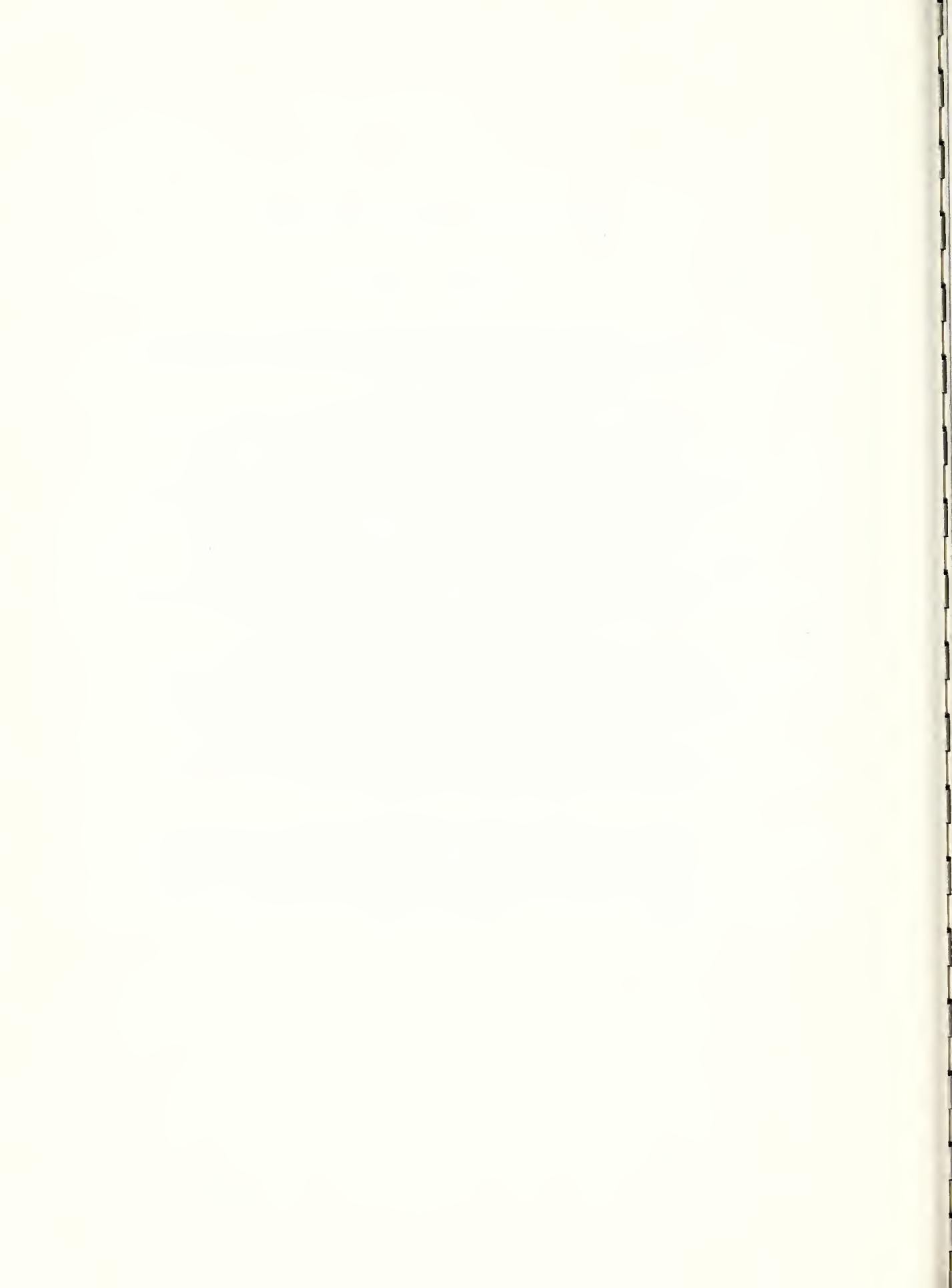
M. Huettel, C. Calkins, A. Hill, and J. Young

Objective: To determine whether adult plum curculio, Conotrachelus nenuphar (Herbst), laboratory reared on apples for many generations can survive and reproduce in the field.

Methods: A wild plum thicket on the Tall Timbers Research Station, Leon County, Florida, was chosen for this experiment. Samples of infested fruit were taken in March and April 1974. The frequencies of alleles segregating at the isocitrate dehydrogenase (IDH) enzyme locus were determined for the samples of larvae by starch gel electrophoresis. In late April, 16 male and 23 female mature, mated plum curculios were released into the thicket. These individuals were from a strain in which the frequency of a naturally rare IDH allele (Idh-S) had been increased by selection (see 53 73(7-12)). Twenty days after the release, the thicket was sampled again and the larvae were electrophoresed.

Results: Analysis of the frequency data from this study indicates that the effective proportion of the released curculios in the resultant population was 0.13. This indicates good survival and reproduction of the inbred lab strain in the field. Making certain assumptions about the equality of the two populations (native and released), we may calculate a native population size of about 260 reproductive adults in the thicket at the time of the release.

Plans: The Idh-S lab strain is being made completely homozygous for this allele. Virgin males and females will be released in separate replicated thickets at bloom time in the spring of 1975. Further analysis of the performance of this lab strain in the field will be made on this basis.



Hibernation Studies of the Plum Curculio,  
Conotrachelus nenuphar (Herbst)

C. Calkins and M. McKoy

(Continuation of report 55 73(7-12))

Objectives: To determine the overwintering mortality and the date of active emergence from hibernation quarters for plum curculios in Florida.

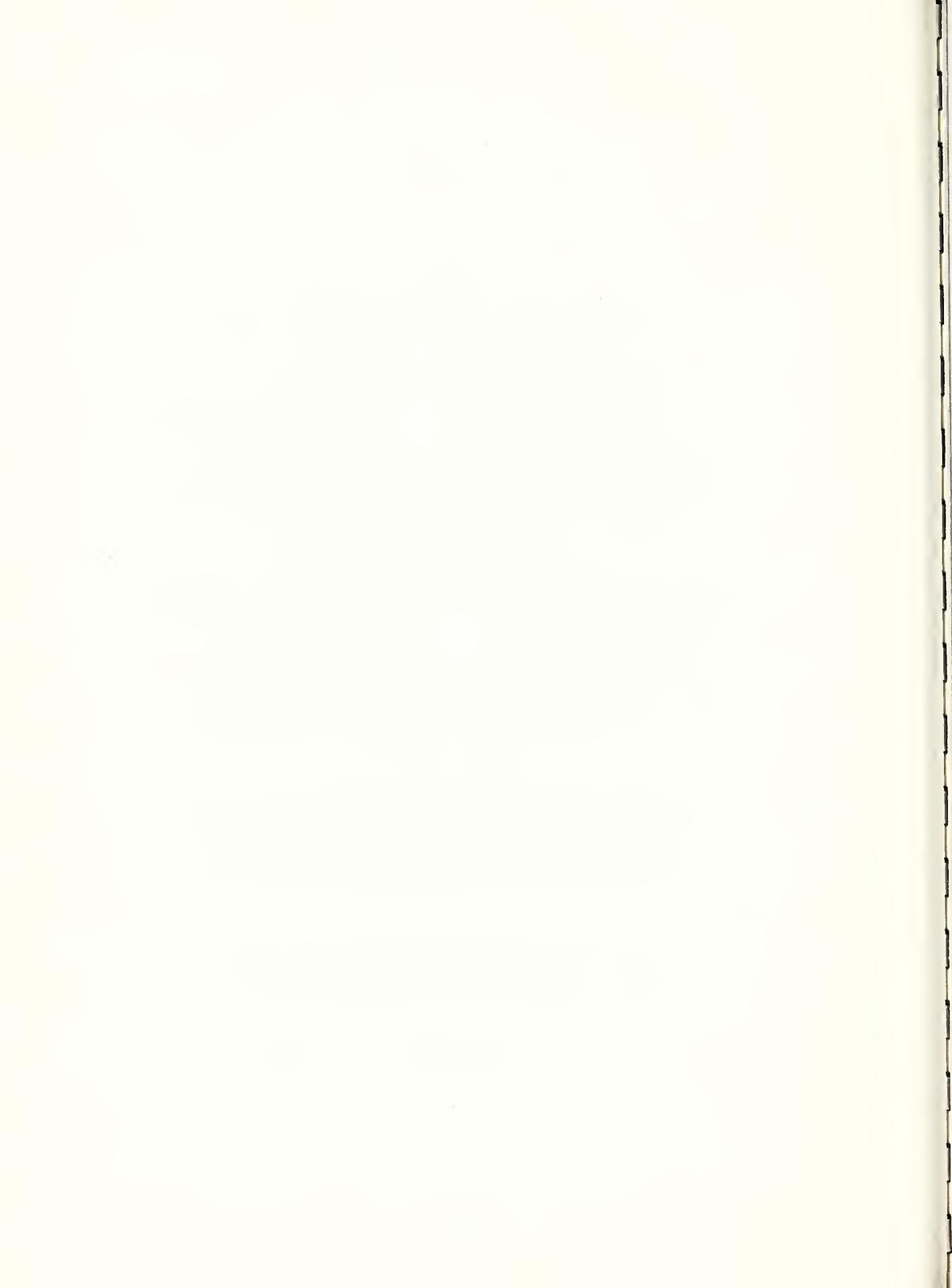
Methods: Plum curculios, reared under semi-outdoor conditions for 2 generations, were placed in 5-gal screened containers at 3 locations in Florida: Quincy, the Tall Timbers Research Station, and Gainesville, Nov. 21, 1973. Cans containing 50 beetles each along with soil and associated plant debris were placed in well protected wooded areas and in more open orchard situations at each location. Weekly observations of beetle activity at Gainesville were made throughout the winter. Monthly collections at all locations were made to determine mortality throughout the winter.

Results: Emergence of adults from detritus within hibernation cans was recorded January 21 and 22 at all locations. Mortality throughout the winter is shown in Table 1. There was less mortality in containers in open areas than there was in sheltered areas at all locations. Maximum-minimum thermometer readings from within the cans indicated that temperature extremes were greater in the open. In light of this, the differential mortality is difficult to explain. There may have been a difference in soil moisture or relative humidity that contributed to mortality.

Plans: Mortality and emergence dates will be determined along with environmental measurements to determine the effect of winter weather on overwintering success and spring emergence. This study will be continued in the future with emphasis upon mating behavior relative to hibernation and the environmental conditions associated with it.

Table 1. Percentage of beetles recovered alive from hibernation containers at Gainesville, Quincy and Tall Timbers Research Station, Fla.

	<u>Gainesville</u>	<u>Quincy</u>	<u>Tall Timbers</u>
Open area	76	65	55
Wooded area	51	51	51



Life Table Studies of Plum Curculio,  
Conotrachelus nenuphar (Herbst)

C. Calkins, A. Hill, and M. Huettel

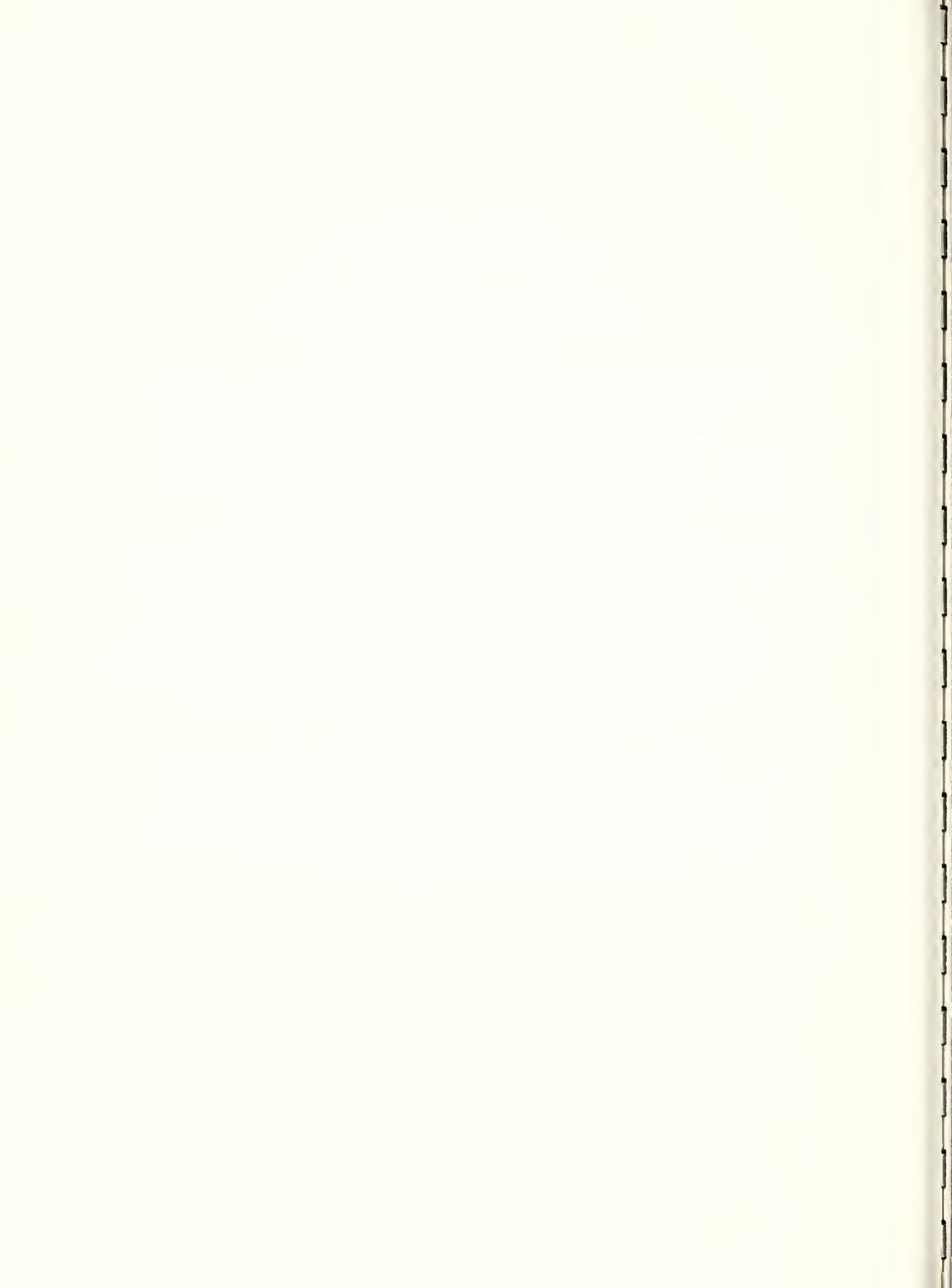
(Continuation of report 73 73(1-6))

Objectives: To determine the innate potential for population growth of plum curculio and the mortality factors involved with each stage.

Methods: Twenty-five pairs of newly emerged adults were isolated separately to determine their mating requirements, fecundity, length of larval development and total adult life span.

Results: Eighty-four percent of the pairs were successful in producing progeny. The mean number of mature larvae per female was 77.9. It took 21 days for mature larvae to appear after initial mating took place. Males and females were observed in copula frequently during the experiment. Females oviposited viable eggs for a maximum of 35 days after the male had died. This may indicate that frequent remating is not necessary for continuation of viable egg production. The life span of females averaged 90 days with a maximum life of over 150 days for 4 individuals.

Plans: Efforts will be made to determine the result of single and multiple matings on fecundity and the total life span of males. Mating experiments with genetically marked strains will be conducted to determine if successive matings supercede each other (that is, do the sperm of later matings mix with sperm already in the spermatheca or do they segregate).



Attraction of Plum Curculios, Conotrachelus  
nenuphar (Herbst), to Their Host

C. Calkins, M. Huettel, and M. McKoy

Objective: To determine the factors involved in the attraction of plum curculios to oviposition sites.

Methods: Attraction of apples as oviposition sites for wild plum curculios was measured by hanging immature apples at 0, 3, and 6 ft above the ground in plum thickets, and in non-host trees about 100 yd from plum thickets. Each treatment was replicated 10 times. Fruit was replaced twice weekly and brought into the laboratory for examination for oviposition scars and held for subsequent emergence of mature larvae.

Results: The test was not initiated until several oviposition scars were observed on plums in the thicket. This assured us that a population was present. Apples placed at ground level were discovered and removed regularly by some animal (probably a raccoon or a opossum), so this treatment was not analyzed. March 15 to April 30 was the time period when plums were present (Table 1). These competed as oviposition sites with the introduced apples. After the plums disappeared, there was a higher rate of oviposition in apples in the plum thicket. No such increase occurred in the non-host area. Apples collected after June 7 showed a very large increase in the number of larvae present. It appeared that this was a new generation emerging. Large increases occurred in both the host and non-host areas. Table 2 indicates that there was slightly more oviposition in apples at 6 ft than there was at 3 ft; however, the differences were not statistically significant. This study indicates that beetles can locate potential oviposition sites on trees which are not hosts, even locating fruit which they normally are not exposed to.

Plans: Larvae emerging from the apples were frozen and will be analyzed by starch gel electrophoresis to determine genetic variability. This may indicate whether one or only a small number of females were responsible for the number of eggs in each area. Additional plans for investigating the orientation of this insect to its hosts involve the use of shape, color, odor, reflectance, etc.

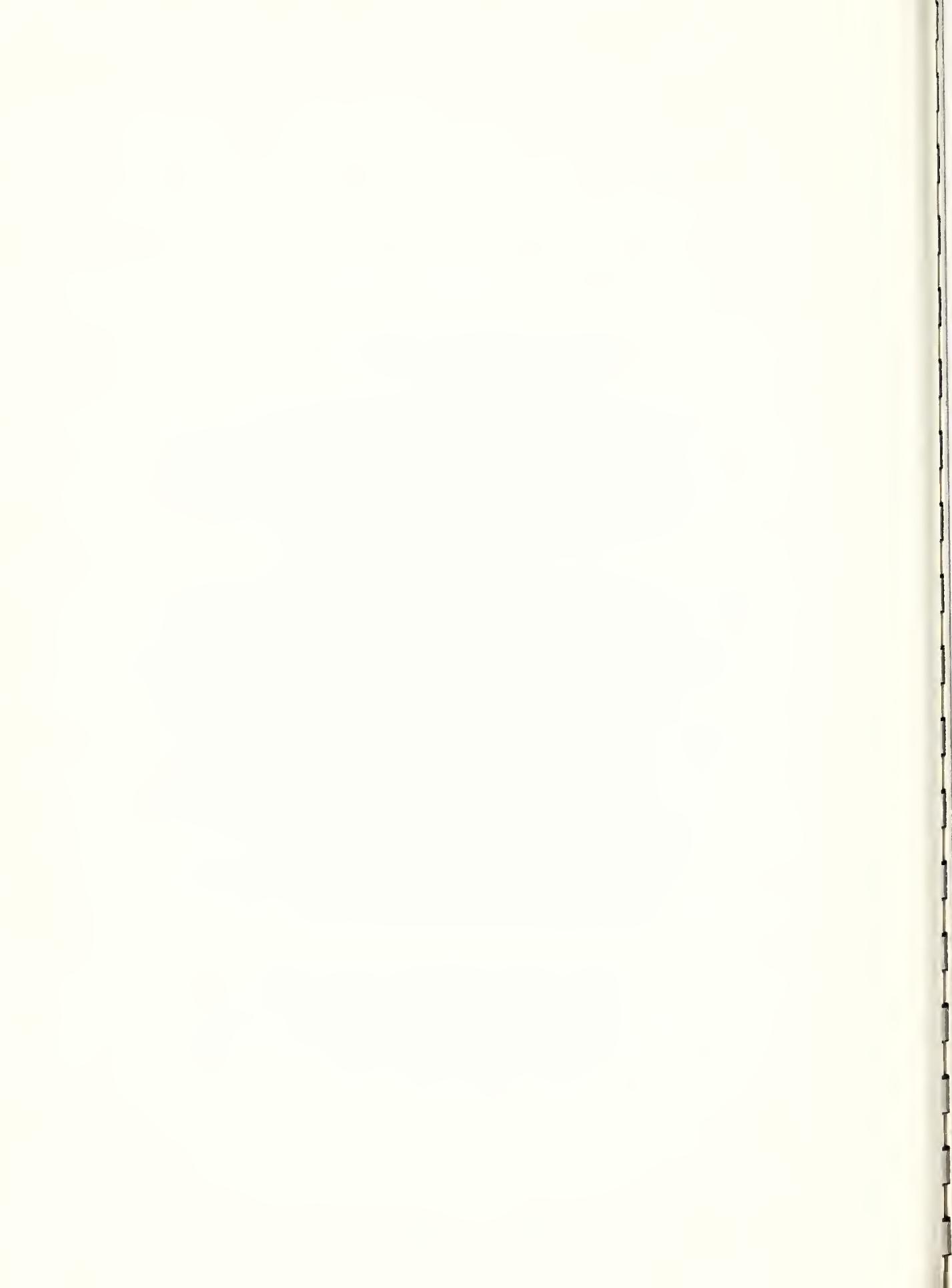
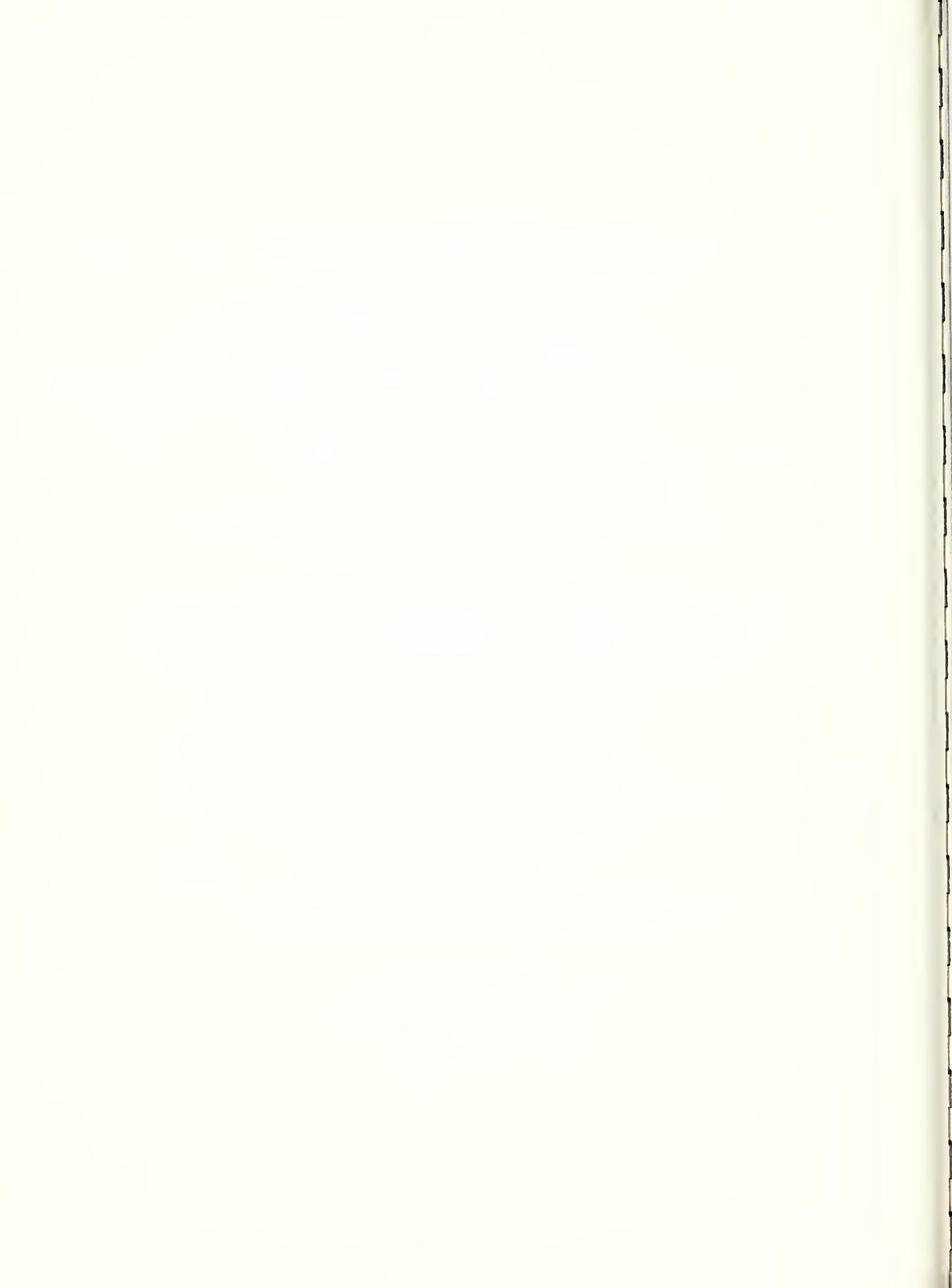


Table 1. The average number of larvae emerging from apples each week during 3 periods from host and non-host areas.

Test area	No. larvae/apple/week		
	Mar. 15 to Apr. 30 (Plums present)	Apr. 30 to June 7 (Plums absent)	June 7 to June 18 (Plums absent)
Host area	1.74	2.36	11.30
Non-host area	.93	.99	7.13

Table 2. The average number of larvae emerging from apples suspended at 3 and 6 ft above the ground in host and non-host areas.

Test area	No. larvae/apple/week		
	(Height above surface, ft.)		
	0	3	6
Host area	0	2.68	3.57
Non-host area	0	1.59	2.20



Seasonal Reproductive Capabilities of Mucidifurax raptor and  
Spalangia endius, House Fly Parasites, in Florida

F. C. Tingle and E. R. Mitchell

(Continuation of report 56 73(7-12))

Objectives: To determine the seasonal reproductive capabilities of 2 species of house fly parasites, Mucidifurax raptor Girault and Sanders, and Spalangia endius Walker.

Methods: Parasite cultures were maintained on lab-reared house fly pupae. Test insects were held in 3 bioclimatic chambers with variable programming for temperature and photoperiod cycles. Mated host-fed female parasites were exposed to either 20 or 40 house fly pupae, 24 h old, for 24 or 48 h. Pupae were either exposed and held at one of the seasonal conditions, exposed at the seasonal condition and held at 26.8°C, exposed at 26.8°C and held at the seasonal condition, or exposed and held at 26.8°C. Each treatment was replicated 10 times. Emerging parasites were counted and sexed 3-5 times/week.

Results: Fewer house fly pupae were parasitized at lower temperatures, and the developmental period of immature stages was longer at higher temperatures. A relatively high percentage of pupae (42-77%) was parasitized by either species when fly pupae were exposed at 26.8°C and then held at any one of the 3 seasonal conditions. There was less parasitism (5-46%) when house fly pupae were exposed to parasites at the lower temperature conditions (spring/fall and winter) and then held for development at 26.8°C. When pupae were exposed at the seasonal conditions and then held at 26.8°C, 8-60% were parasitized, and when held at the same seasonal condition as exposed, 11-67% produced parasites.

The developmental time and emergence periods, as well as the % parasitism, for the 2 parasite species tested at the seasonal conditions are shown in Fig. 1. S. endius took somewhat longer for development at all temperatures. The wild culture of M. raptor emerged over a slightly longer period than did the lab culture at summer conditions, but emergence began at ca. the same time. Under spring/fall conditions, the wild culture of M. raptor required less time to develop than did the lab culture, and developed much faster than the lab culture at the winter conditions.

Plans: This research has been completed.

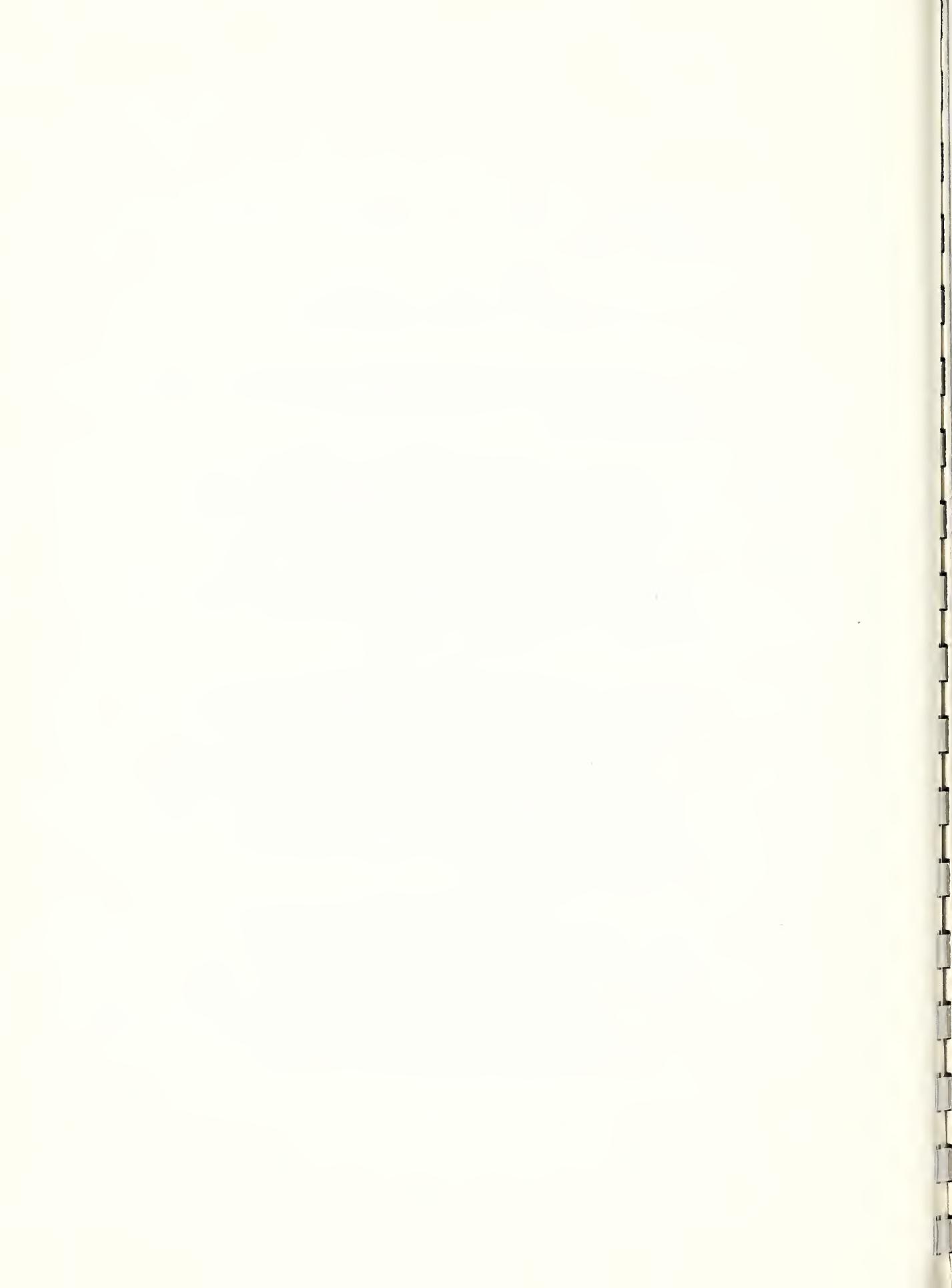
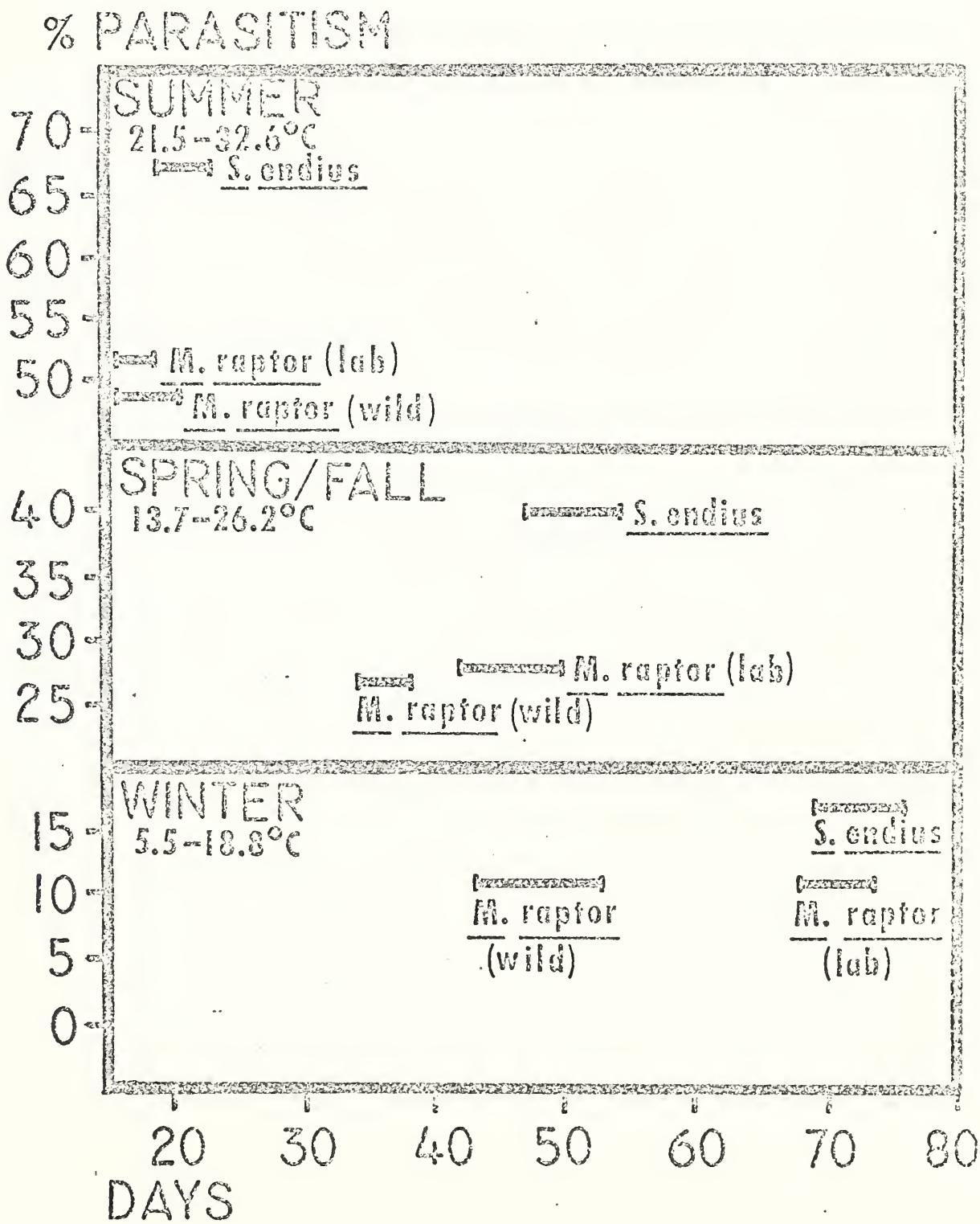
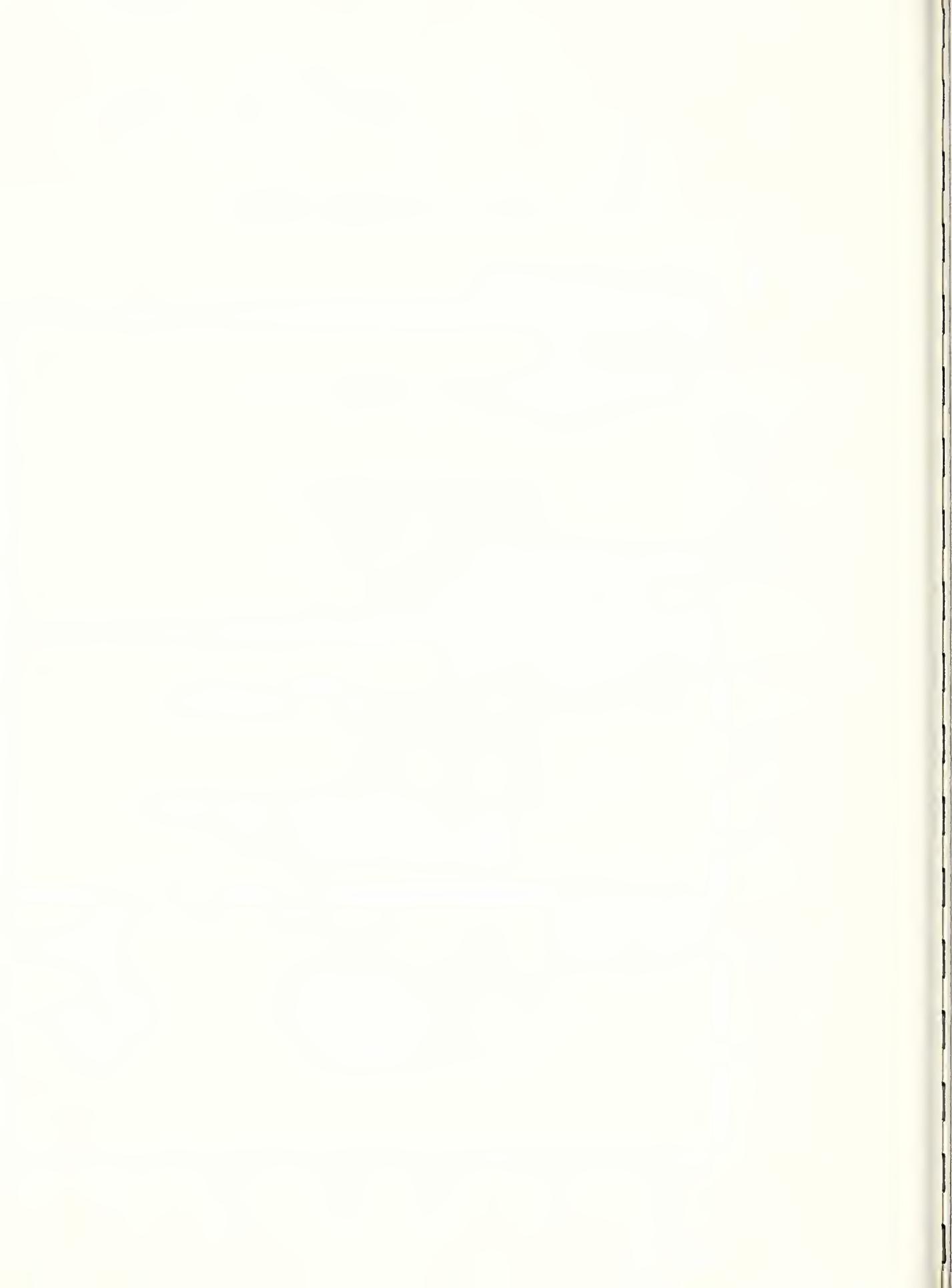


Fig. 1. Percent parasitism and developmental time for 2 species of parasites, Mucidifurax raptor and Spalangia endius, on house fly pupae, 1 ♀ parasite/40 pupae, at indicated seasons, Gainesville, Florida.





Peachtree Borer Electric Grid Trap

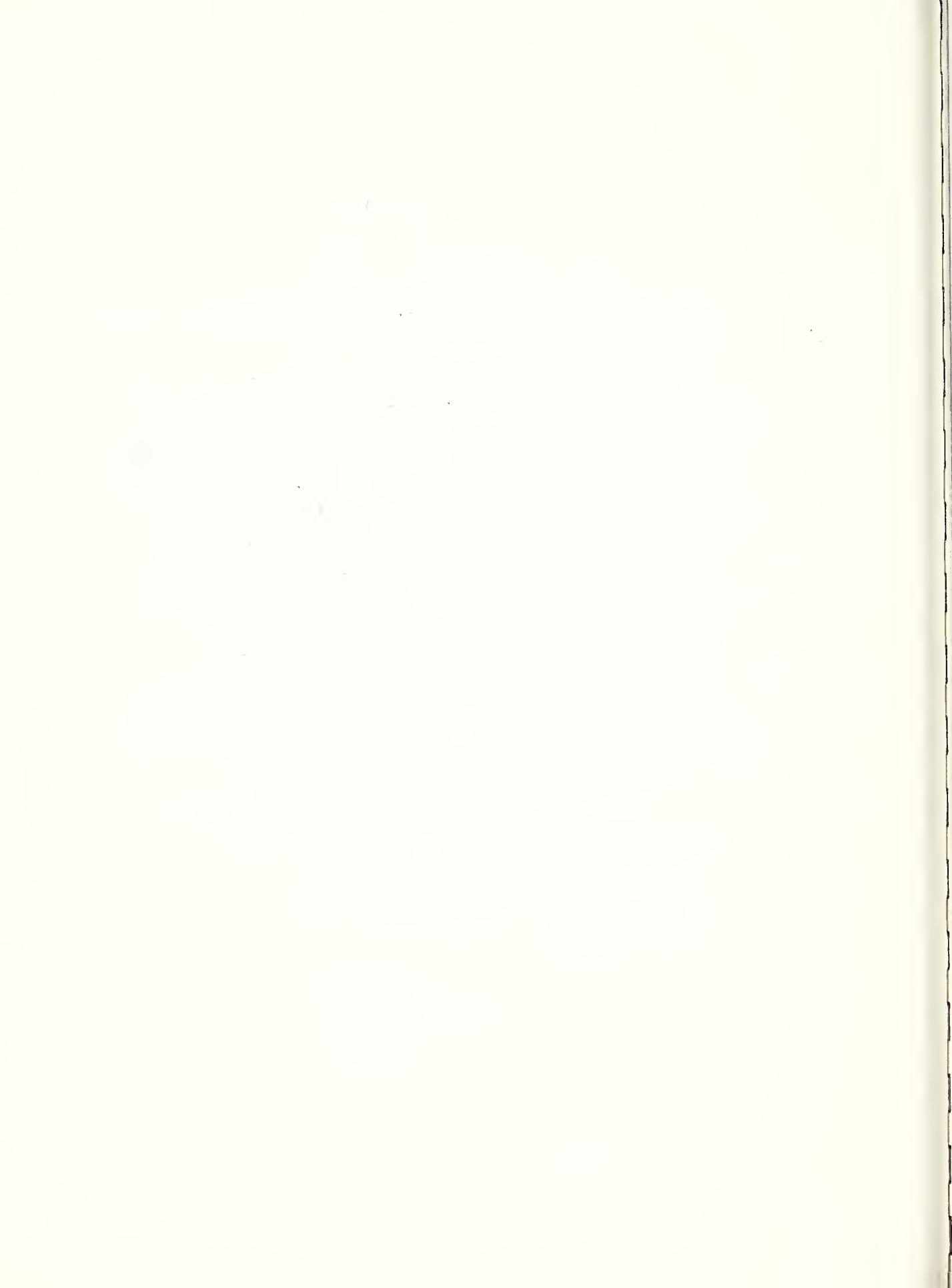
E. W. Hamilton and J. Brogdon

Objective: To design an electrocutor-type trap for collecting lesser peachtree borer adults.

Description: The electrocutor grid was constructed of 1" hardware cloth formed in 2 cylinders. The outer cylinder, ca. 12" diam, is connected directly to a sheet metal funnel leading to a collecting jar. The inner hardware cloth cylinder is ca. 11" diam and is held in place inside the outer cylinder by 6 spacers made of 1/4" plexiglass. HV power leads are connected one to the outer and the other to the inner wire cylinder. The HV supply consists of a 12 VDC to 15,000 volt converter and draws 100 ma current. A small 12 VDC motorcycle battery powers the unit (9 AM - 1 PM) for about 1 wk on a charge. A light activated switch, similar to that described previously (63 73(7-12)), controls the on time of the trap. The trap is designed to be hung in a tree.

Results: Calling females were used as the attractant source. Preliminary tests indicate good results in capturing lesser peachtree borer males, 57 males being the maximum number caught in one day. The grid design of the trap prevents accidental shocks because only the outer electrode is exposed to the touch. The trap is relatively simple and inexpensive to construct.

Plans: Tests on the amount of time required for maximum effectiveness and on trap configuration will continue.



Trapping Equipment for the Almond Moth,  
Cadra cautella: Evaluation of Trap Design

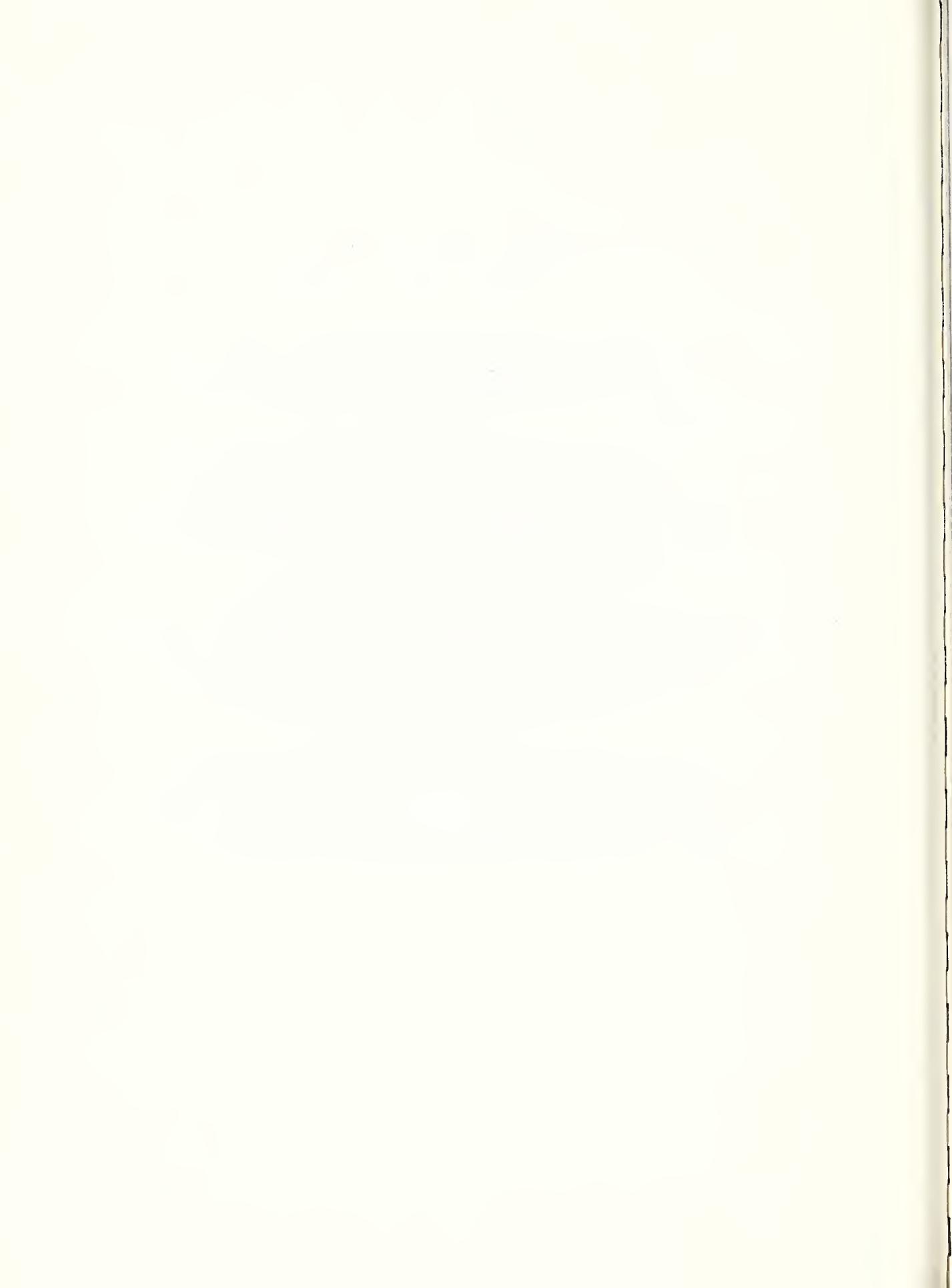
J. M. Stanley and D. W. Hagstrum

Objectives: This study has been initiated to design an effective trap for collecting the adult of this insect in stored product warehouses and to evaluate the potential of such equipment in insect management.

Methods: This nocturnal moth has been found to be positively attracted to electromagnetic radiation. Several existing electric insect traps and specially designed units were tested in laboratory rooms to determine their effectiveness in attracting and trapping almond moths. Procedures were the same as those for evaluating biological parameters.

Results: Small traps were found to be more satisfactory for use in warehouses than larger traps designed for field use. Preliminary results indicated that lamps with relatively lower energy output offer more promise than intense sources. A satisfactory time interval trap for warehouse use was designed, constructed and tested.

Plans: Further trap designs will be constructed and tested. Traps showing promise will be tested in citrus pulp and peanut storage warehouses where wild populations exist. Consideration will be given to testing those traps that show potential effectiveness in attracting and trapping moths for population suppression capabilities.



Trapping Equipment for the Almond Moth, Cadra cautella:  
Evaluation of biological parameters

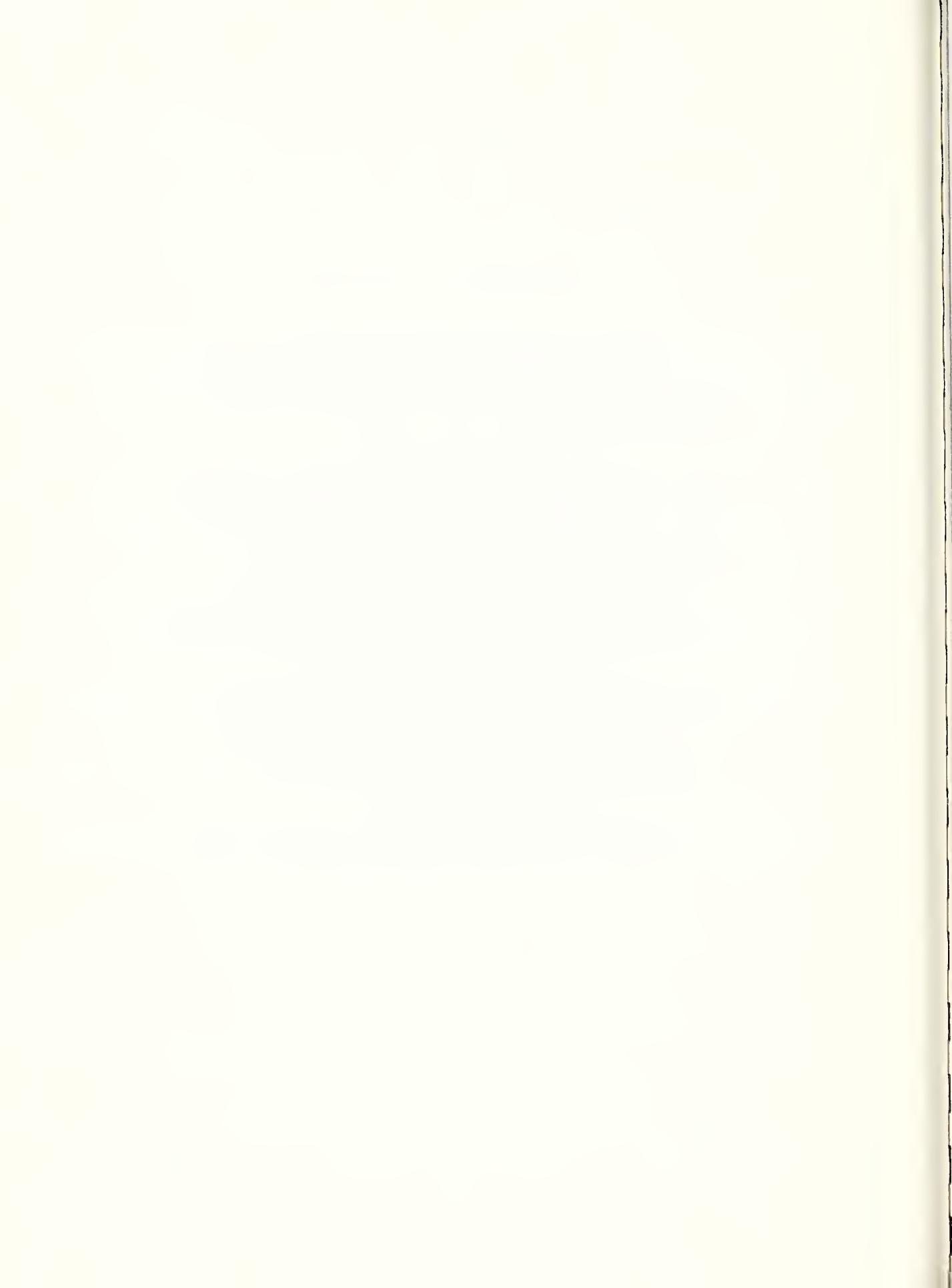
D. W. Hagstrum and J. M. Stanley

Objective: To determine whether biological parameters such as sex, age and density of adults, or time of day have a significant effect upon the efficiency of traps. This information will be useful both in further development of trap design and the eventual utilization of traps in insect management programs.

Method: Light traps were operated in the center of several 20' x 20' rooms. The age, sex ratio (1:1), and number of moths released in the rooms were known. The number of moths caught and the time of day when they were caught were determined during 2 successive 24-hr periods following release. The photoperiod used for rearing the moths was shifted to determine whether this procedure would result in a similar shift in the time of day that the moths were caught. Tests were also made in a citrus pulp warehouse to determine whether the results obtained there would be comparable with those obtained in the rooms.

Results: The moths in the rooms were caught in greatest numbers during the evening and in the warehouse during the early morning. Fewer males than females were caught, but differences were not statistically significant. Collection of females before oviposition occurs would be particularly important in an insect management program. About 35% of the population was collected regardless of age.

Plans: All tests will be replicated further. In particular, more tests on the effects of density and shifted photoperiod are needed.



Trapping the Beet Armyworm, Spodoptera exigua

F. C. Tingle, E. R. Mitchell, and W. W. Copeland

Objectives: To determine the most effective traps available for capturing the beet armyworm (BAW); also, to determine their time of activity.

Methods: BAW ♀♀ (3/trap) were used as the attractant in traps of 6 designs (an electric grid trap and 5 non-power traps) for capturing BAW ♂♂. Traps were baited with fresh ♀♀ and checked every 2 or 3 days. The effect of color on moth catch was also evaluated. A time-interval electric grid trap was used to determine the time of activity.

Results: The electric grid trap captured ca. 5 times more BAW ♂♂ per night than the Pherocon 1C sticky trap. The double cone trap captured significantly fewer moths than any of the sticky traps; there was no significant difference in catch among any of the sticky traps (Pherocon 1C, Sectar XC-26, Sectar I and pink bollworm trap).

Fifty collections were made with the time-interval electric grid trap May 15-July 17, and a total of 19,224 BAW ♂♂ were captured. Of these 92% were caught between midnight and 6 AM.

Trap color apparently had little effect on catch. Colors tested were fluorescent orange, red, and yellow; white; black; silver; green; and international orange.

Plans: This information will be used in evaluating chemical attractants and measuring beet armyworm populations.

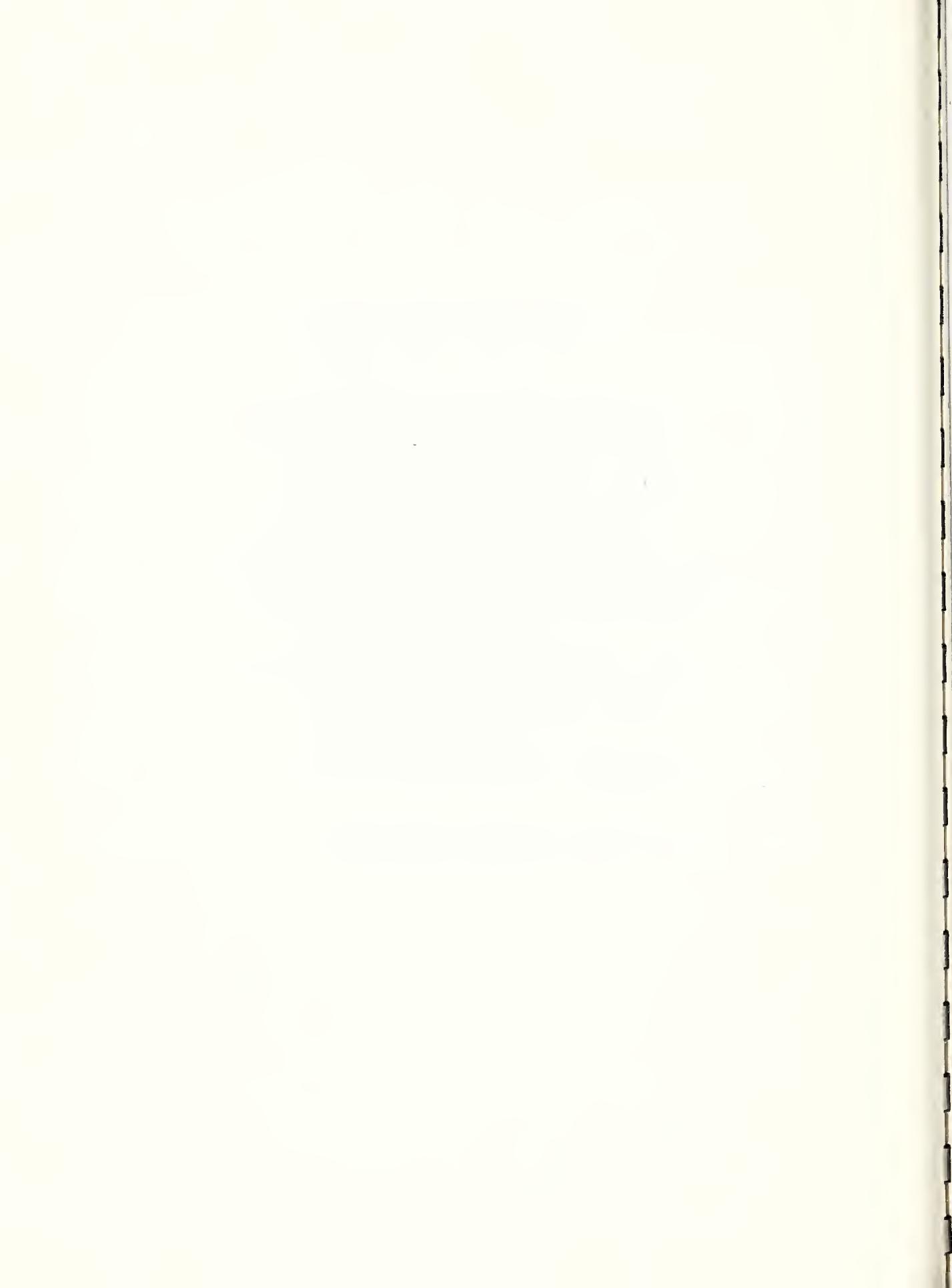
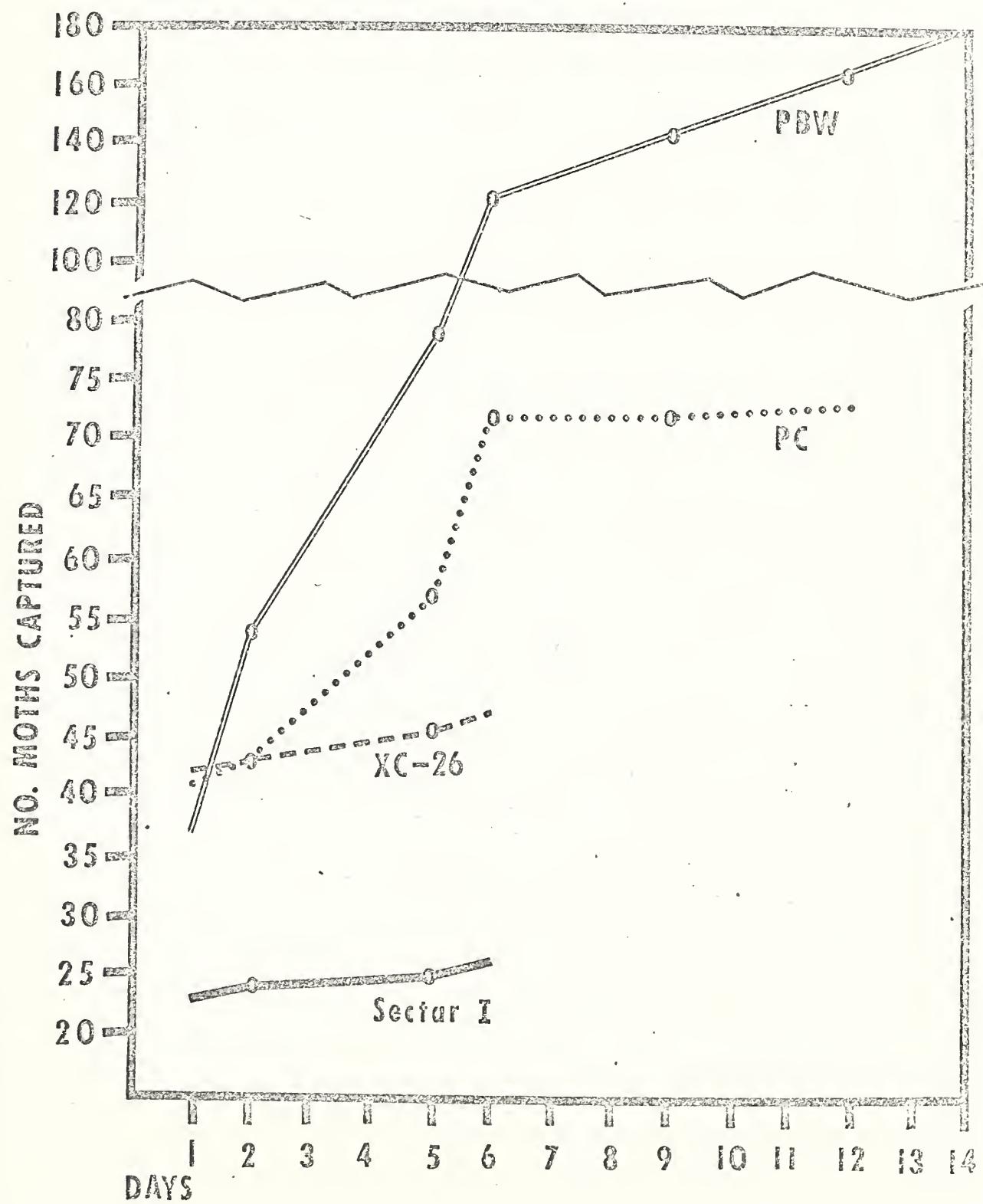
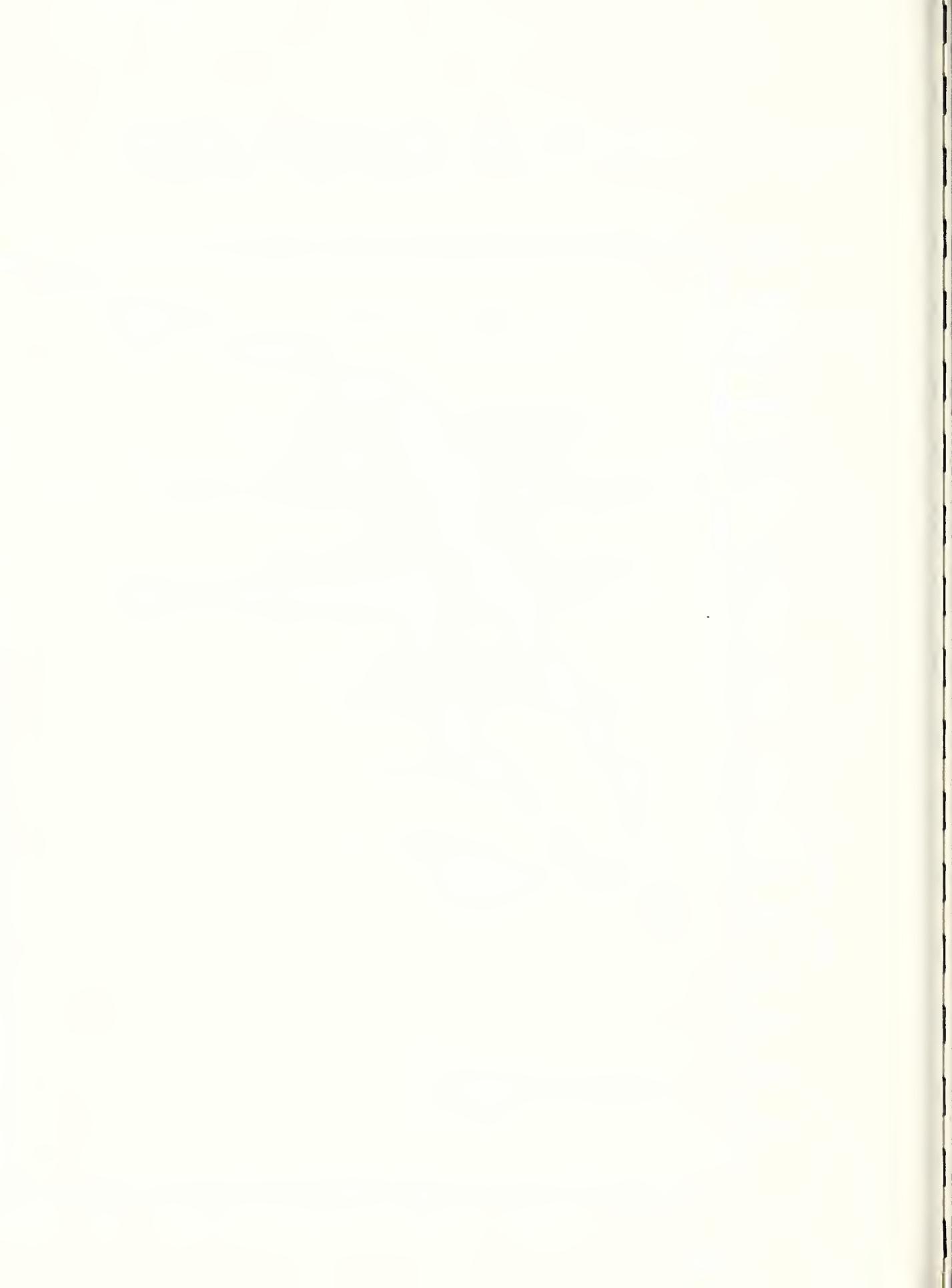


Fig. 1. Cumulative mean number of male fall armyworm moths captured on indicated traps (3 replicates each): Pherocen 1C (PC), Sector XC-26 (XC-26), Sector I (Sector I), and Pink Bollworm (PBW). Hastings, Fla.





Trapping the Fall Armyworm, Spodoptera frugiperda

F. C. Tingle, E. R. Mitchell, and W. W. Copeland

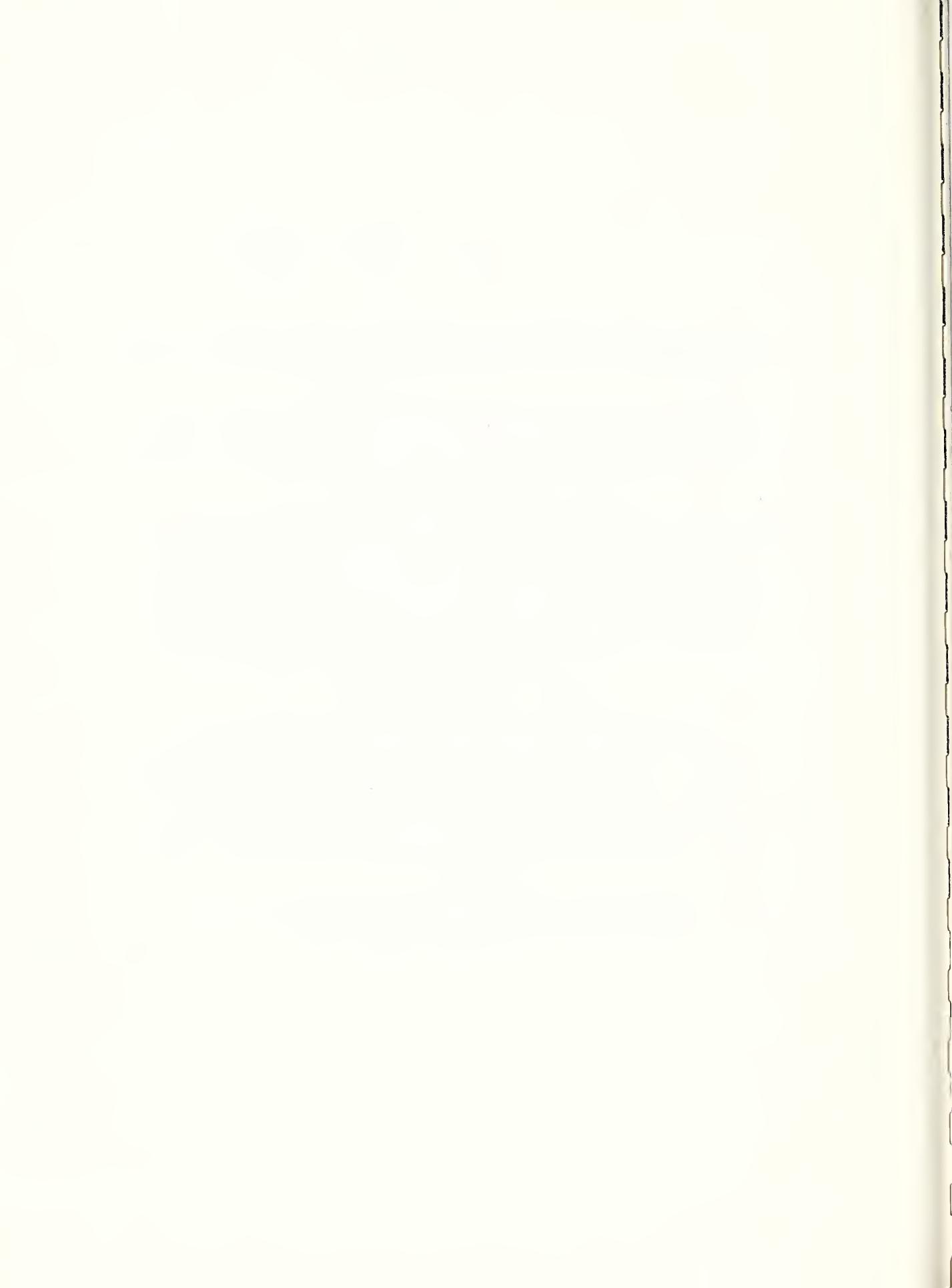
Objectives: To determine the most effective trap available for capturing the fall armyworm (FAW); also, to determine the capacity for traps tested.

Methods: The FAW sex attractant Z-9-dodecen-1-ol acetate (Z-9-dda) was used at the rate of 25 mg/trap as the attractant in traps of 7 designs (an electric grid trap and 6 non-power traps). The effect of color on moth catch also was checked.

Results: The Pherocon 1C, Sector XC-26, and pink bollworm sticky traps captured ca. twice as many FAW ♂♂ as the smaller Sector I trap. The double cone trap captured significantly fewer moths than any of the sticky traps. The electric grid trap caught ca. 10 times more FAW ♂♂ than the Pherocon 1C trap. Trap color had little effect on catch. Colors tested were fluorescent orange, red, and yellow; white; black; silver; green; and international orange.

Fig. 1 shows the comparative capacity of the non-power sticky traps. The Sector I trap probably reached its capacity the 1st night before a comparative sample (for 1 night) of the population was captured. The Sector XC-26 trap apparently reached capacity the 1st night although it appears that a comparative sample was captured. The pink bollworm trap, after the 1st night, captured more moths than the Pherocon 1C trap, due to the larger sticky surface area.

Plans: This information will be used in evaluating chemical attractants and measuring fall armyworm populations.



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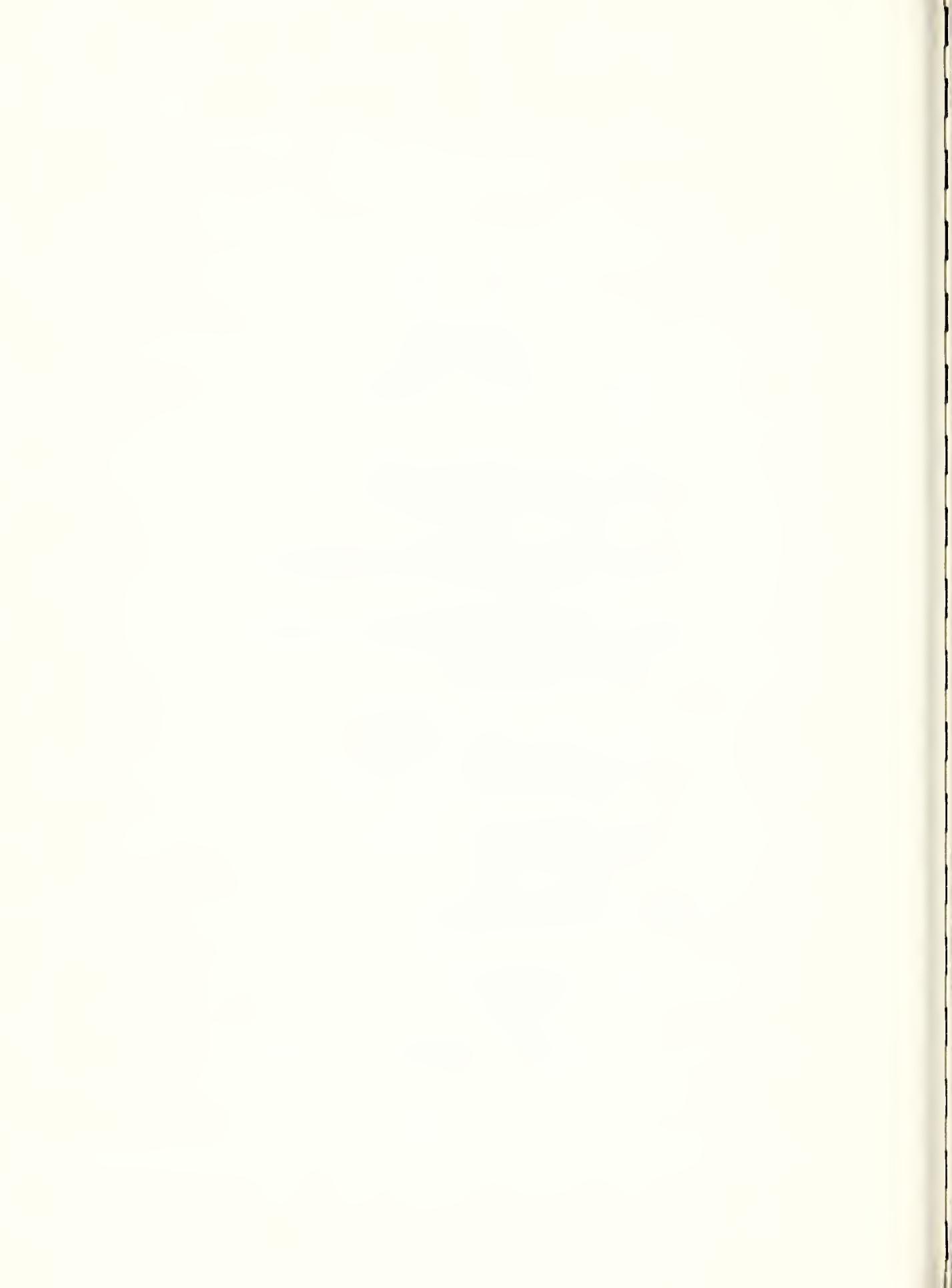
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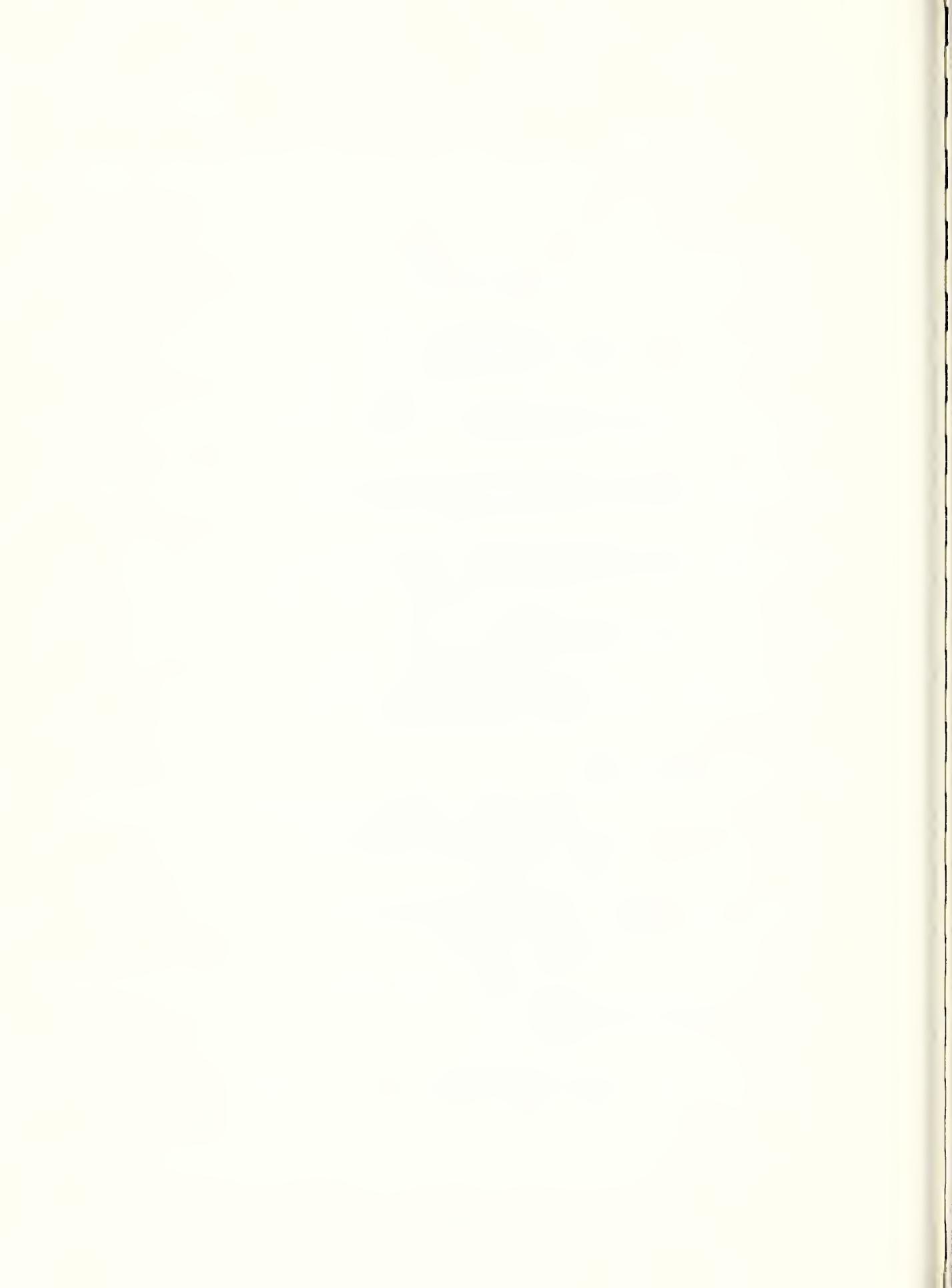
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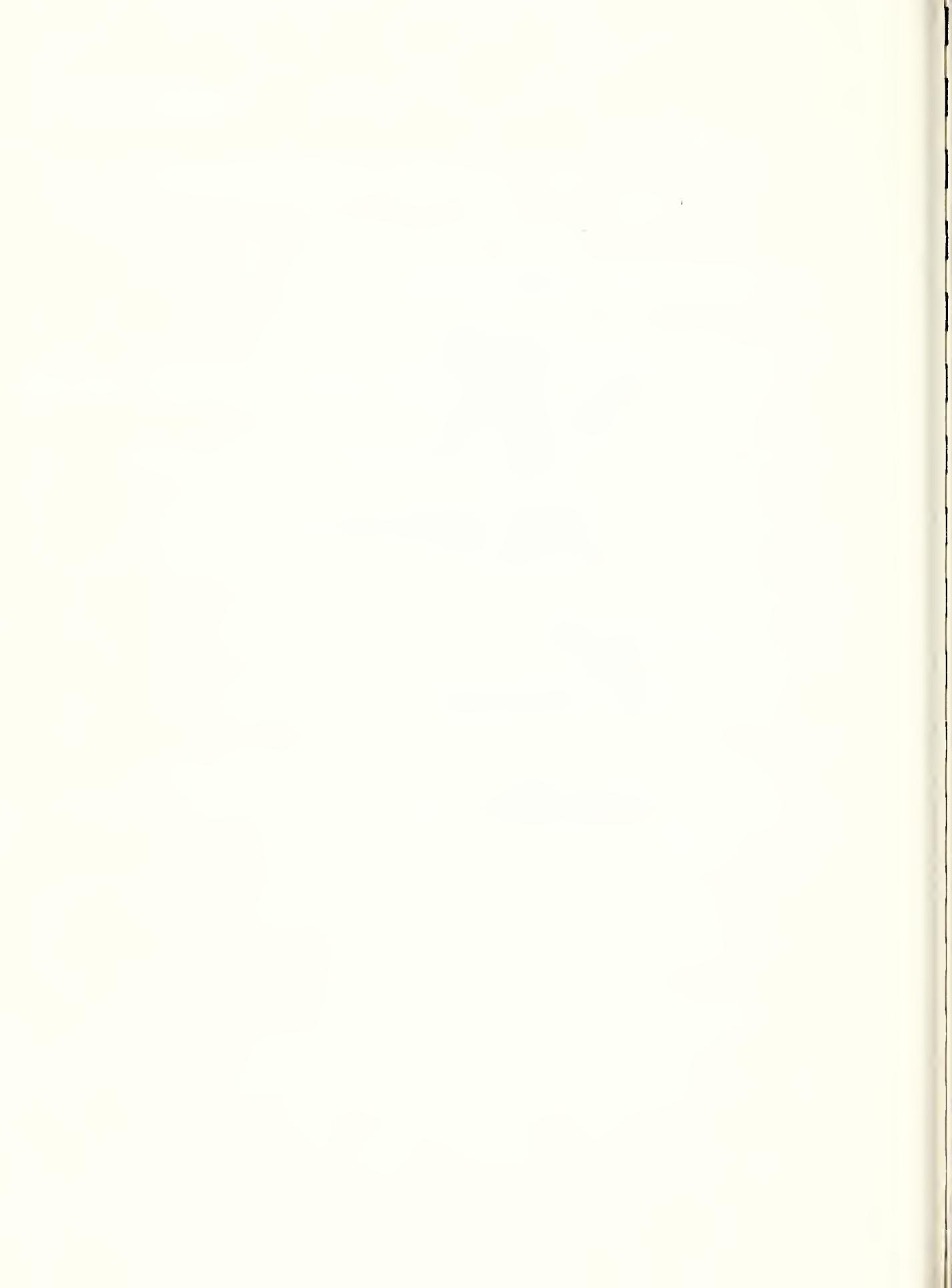
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MANUSCRIPT STATUS REPORT

September 12, 1974

NATIONAL TECHNICAL EDITOR REVIEW\*

Ashley, Tom R. Computer program for analyzing parasite-host or predator-prey relationships.

\*Calkins, C. O. and V. M. Kirk. The distribution of false wireworms (Coleoptera: Tenebrionidae) in relation to distribution of soils in South Dakota.

\*Chalfant, R. B., C. S. Creighton, G. L. Greene, E. R. Mitchell, J. M. Stanley, and J. C. Webb. Investigation of the cabbage looper with sex pheromone-baited blacklight traps in Florida, Georgia, and South Carolina.

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\*Marzke, Frederick O., Sam R. Cecil, Arthur F. Press, Jr., and Phillip K. Harein. Quality and Germination of peanuts stored at various temperatures in high concentrations of nitrogen and carbon dioxide.

Mayer, M. S. Hydrolysis of pheromone by the antennae of Trichoplusia ni.

Mayer, M. S., S. M. Ferkovich, and R. R. Rutter. Specificity and localization studies of pheromone degradative enzymes isolated from an insect antennae.

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\*Sharp, J. L. and D. L. Chambers. Gamma irradiation effect on the flight mill performances of D. dorsalis and C. capitata.

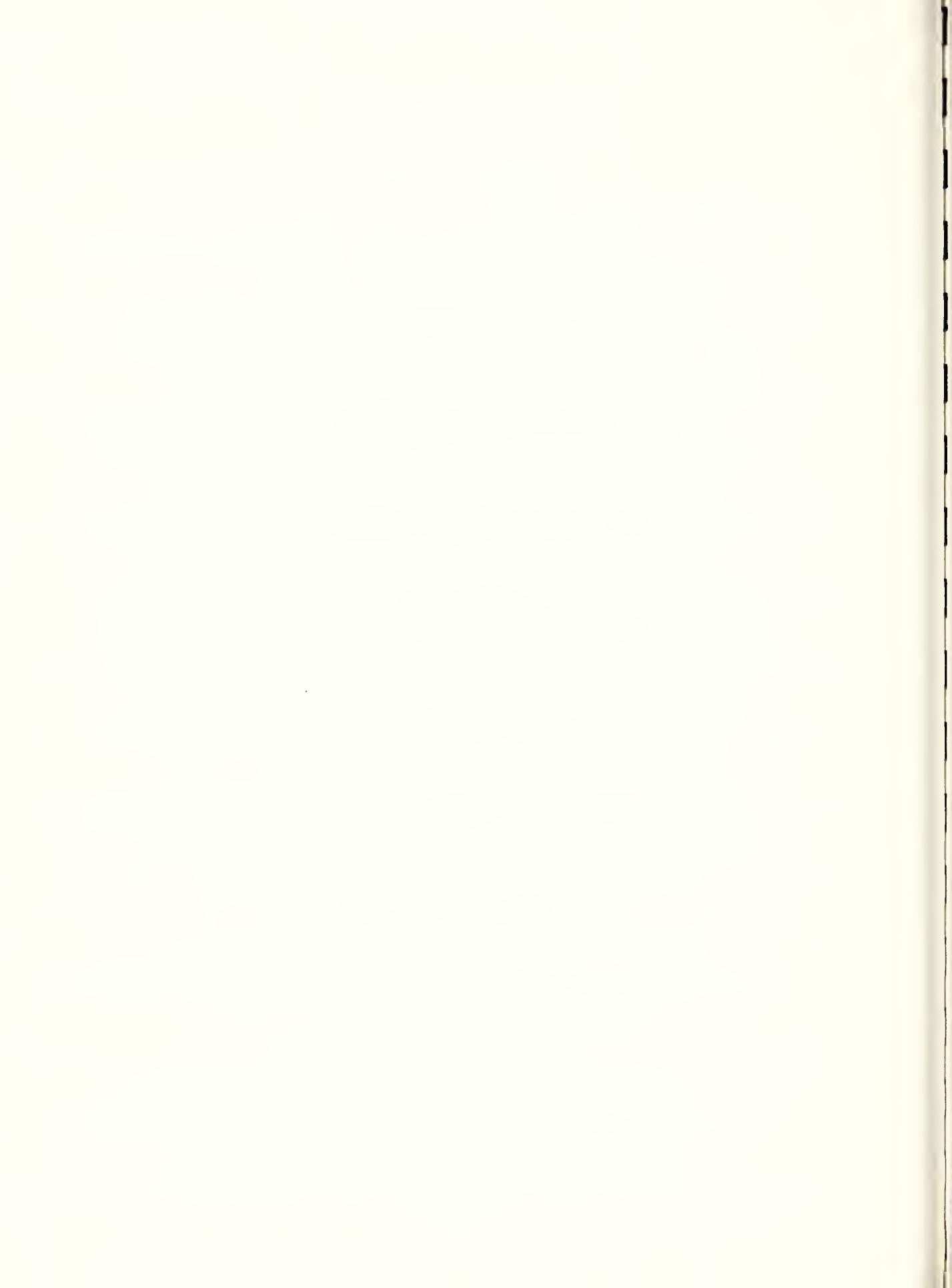
Sower, L. L., W. K. Turner, and J. C. Fish. Reduced mating frequencies among Plodia interpunctella in the presence of synthetic sex pheromone.

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\* Manuscripts having reached this stage are available to laboratory staff, for information only (see Mrs. Blackwell).



Appendix 2 (2)

Callahan, Philip S. The insect antennae: I. Log-periodic infrared emission from the cabbage looper pheromone and its relationship to the log-periodic spacing of the sensilla trichodea of the noctuid antenna. Ann. Entomol. Soc. Am. (Submitted 9/10/74).

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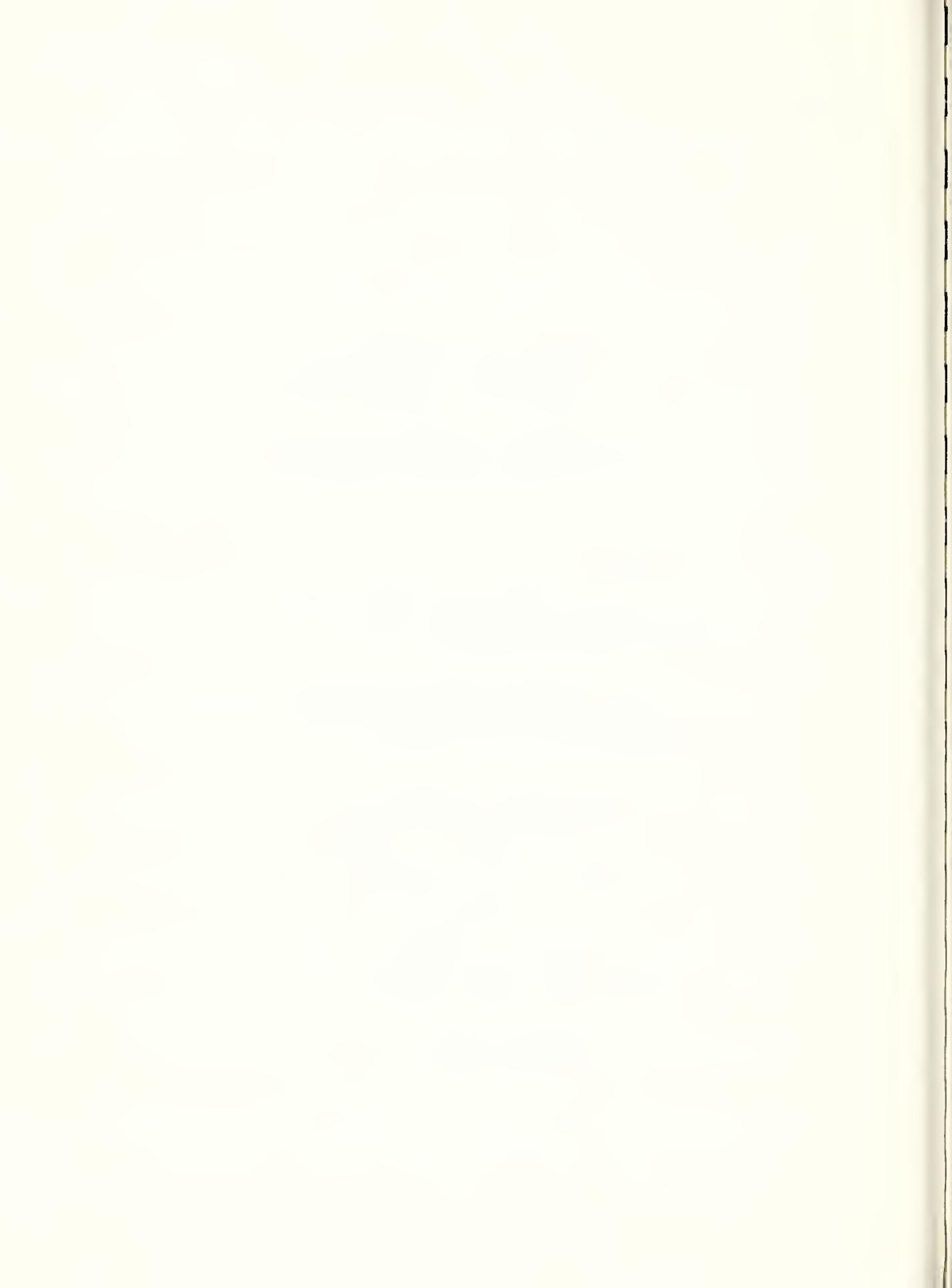
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Appendix 2 (4)

\*Leppla, Norman C., Lonnie N. Standifer, and Eric H. Erickson, Jr. Culturing blister beetle larvae on diets containing different kinds of honey bee-collected pollen. *J. Apicultural Res.* (Accepted 4/74).

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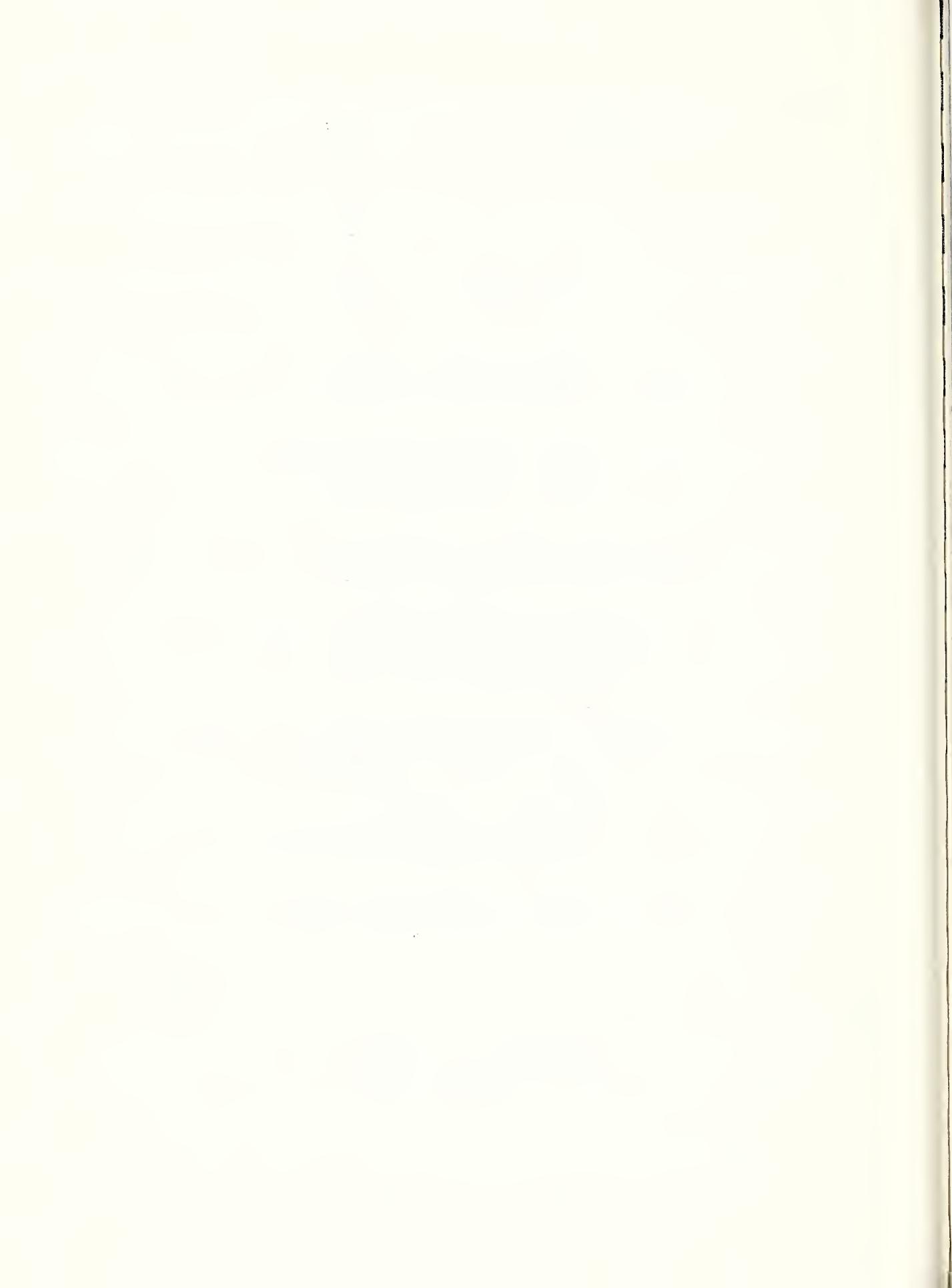
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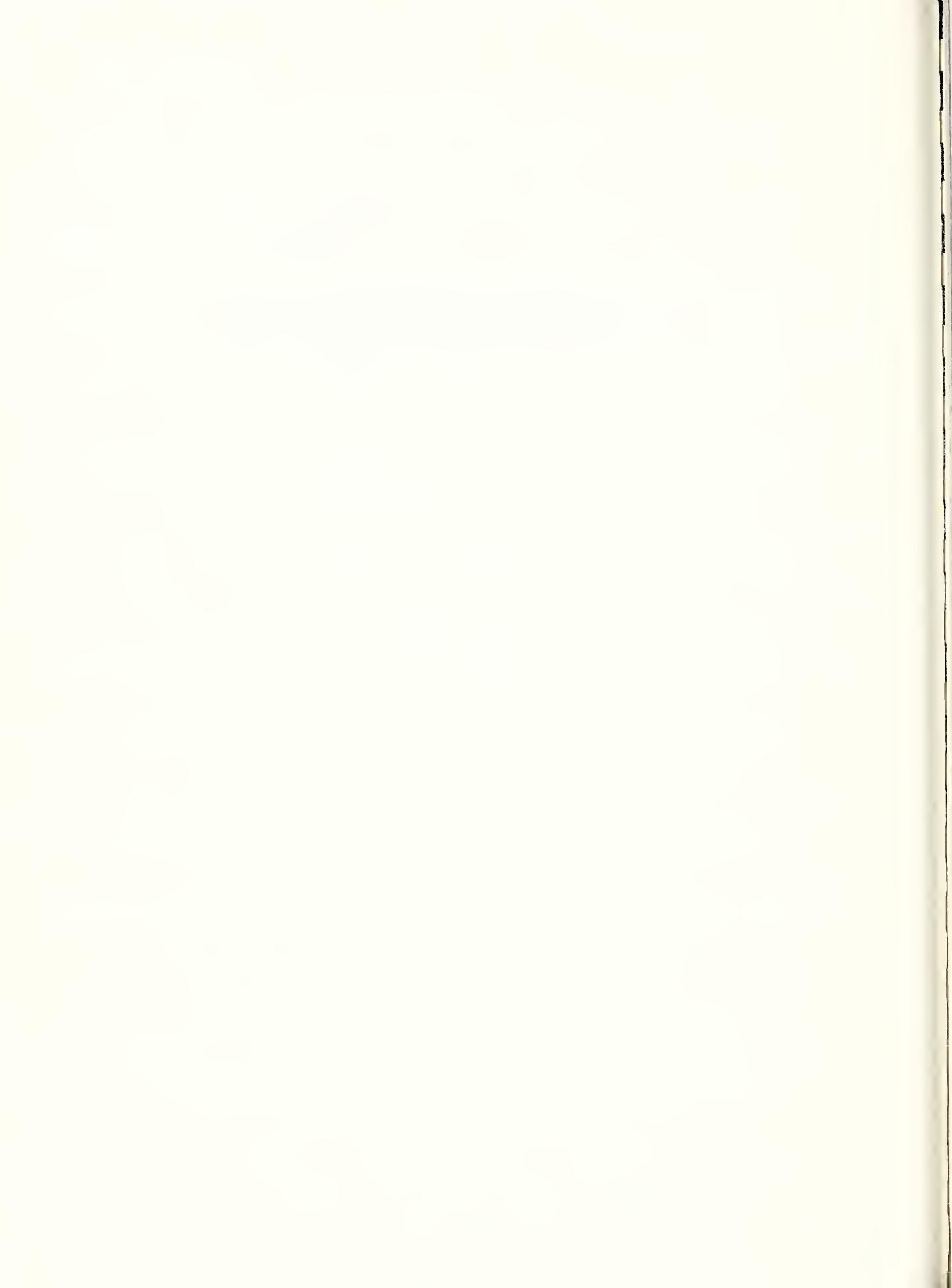


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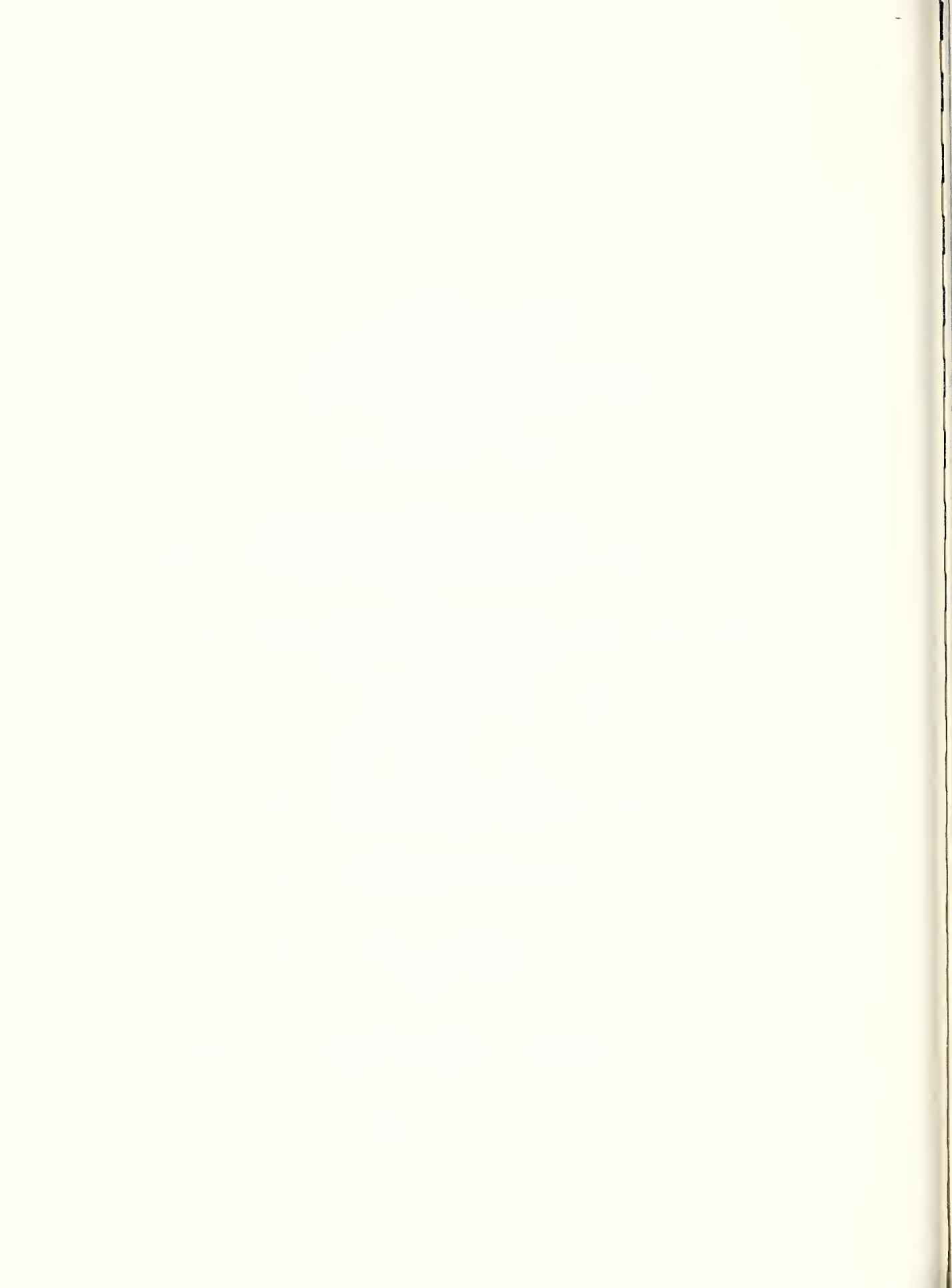
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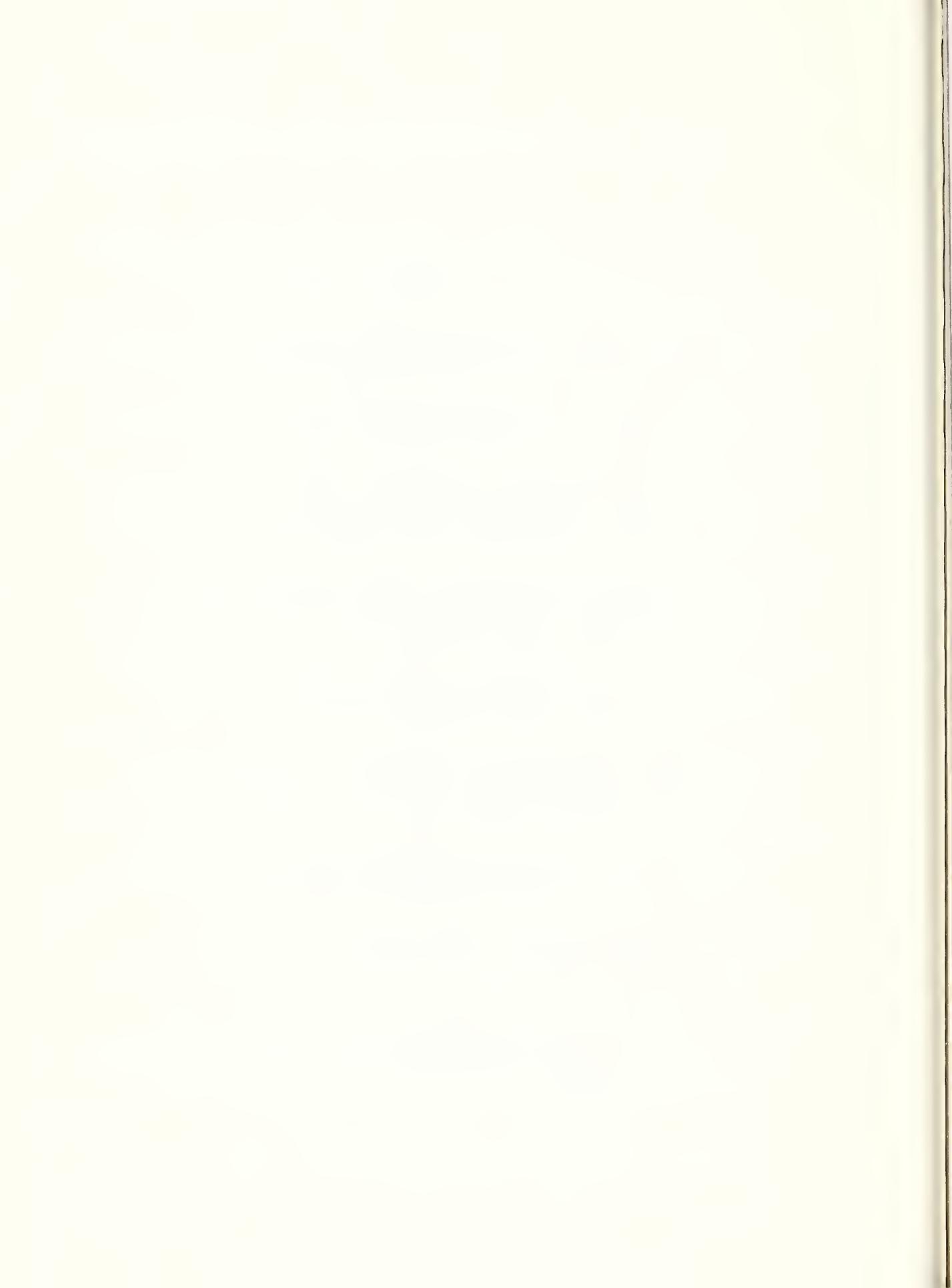
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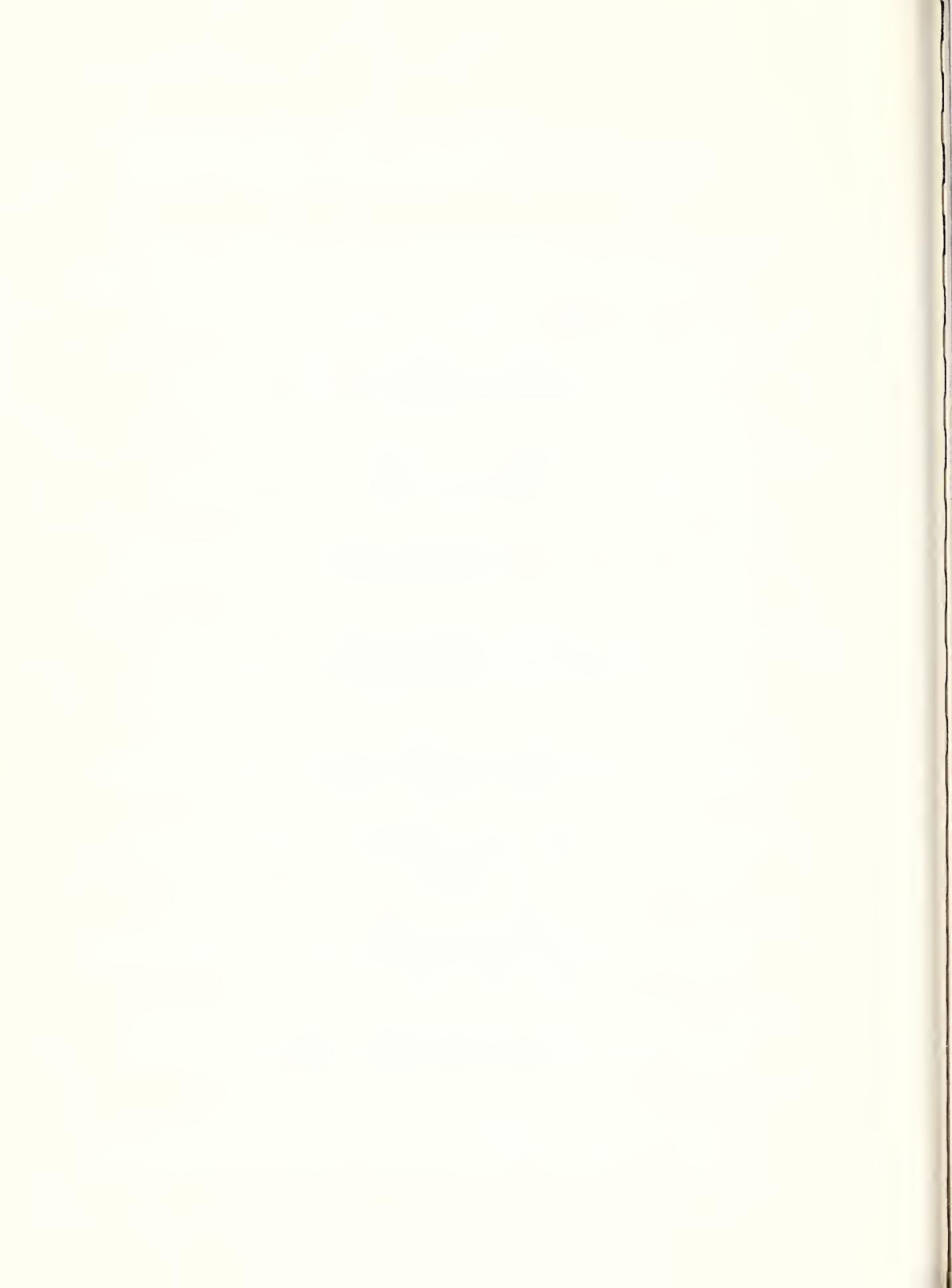
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